ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT

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ANNUAL REPORT

OF

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NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT

July 1, 1971 through June 30, 1972

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NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972

OFFICE OF THE DIRECTOR

In fiscal year 1972 a number of significant events directly affected Institute program and administrative activities:

--recognition by Congress and the public concerning the need for more research on sudden infant death syndrome;

--legislative action by Congress to establish a separate Institute for Aging Research;

--continued restrictions on employment compounded by requirements to reduce average grades;

--increased concern at all levels for providing equal employment opportunity, and for insuring proper representation of minorities and women on decision making bodies;

--significant increases in dollars (18%) and permanent full-time staff ceilings (10%).

Reorganization of Administrative Management Functions

As this Institute was originally structured, administrative management was carried out as a part of the Office of the Director and organizationally was not differentiated from the activities of the immediate staff of the Director. Under this arrangement the organizational and functional statement of the Institute did not include important administrative functions. There was no indication of the line relationship of the Personnel Officer, Budget Officer or Institute Administrative Officer to the Executive Officer and, ultimately, to the Director.

This year an Office of Administrative Management (OAM) was created, separate from the immediate Office of the Director and on an organizational level with the Public Information Office and the Office of Program Planning and Evaluation.

The components of this Office are the Executive Officer and his staff, the Financial Management Office, Personnel Management Office, and the Institute's Administrative Office. Such an arrangement readily allows visibility for these offices, clarifies the reporting relationships, and more clearly defines the responsibility of the Executive Officer.

Late in the year the Reference Services Section was transferred to OAM from the Scientific Information and Publications Branch.

Staffing

As most Federal organizations, NICHD's staffing pattern was primarily dependent upon the employment ceiling constraints imposed upon it. In summary, the total full-time permanent position ceiling rose by forty-five during this fiscal year and the other than full-time permanent ceiling was increased by eight positions. The significant growth area was in the intramural research program with special emphasis on the Gerontology Research Center in Baltimore, Maryland. Unfortunately, the full-time permanent ceiling that was allocated to the Institute changed three times and the other ceiling was altered four times during fiscal year 1972. This resulted in an incremental staffing pattern that taxed management to its limits of staffing and placement efficiency - especially since a significant increase occurred very late in the fiscal year. The following tables summarize NICHD's staffing:

Table 1.A. Full-Time Permanent Ceiling Milestones for FY 72

	7/1/71	11/15/71	3/31/72	5/22/72-6/30/72	Total Net Change
OD	26	30	26	34 1/	+8
OSP	244	264	267	277	+33
PS	75	74 1/	73 1/	73 1/	-2
EP	42	42	42	42	even
CPR	33	37	37	37	+4
EB/OPPE	17	17	17	17	even
IS	8	15 1/	15 1/	10 1/	+2
Total	445	479	477	490	+45

1/ Includes a transfer of function from PS to IS to OD within FY 72.

Table 1.B. Other Than Full-Time Permanent Ceiling Changes.

DATE	Total Ceiling
7/1/71	60
8/9/71	61
11/15/71	57
12/26/71	58
5/22/72-6/30/72	68

Note: (1) At each point in time, 55% or more of these positions were allocated to OSD.

(2) This table does not include the 133 ceiling exempt positions that include: Visiting Fellows, Stay-in-School employees; Management Interns; Guest Workers (65 employees); Guest Scientists, and various College Work-Study programs.

Grade De-escalation

The average grade level reduction constraints followed the sporadic pattern of the Institute's ceiling changes. Although the table below gives a brief statistical summary, the real impact was felt in three areas that tended to lower program productivity: (1) increased administrative overhead to control, adjust, and plan; (2) lower employee morale; and (3) the high turnover rate in lower graded and generally more mobile employees.

Table 2

June 30, 1971 Actual Average Grade adjusted for Summer Program & Stay-in-School	9.0773
January 31, 1972 Actual Average Grade Full-time General Schedule	8.9526
June 30, 1972 Average Grade Goal not adjusted for 1972 Summer Program	8.9526
June 30, 1972 Average Grade Goal adjusted for Summer Program	8.2142
Actual Full-Time General Schedule Reduction not adjusted for Summer Program	0.1247
Fiscal Year 1973 Government-wide Reduction Projection	0.1300

Upward Mobility

Because of the recent promotion freezes, grade level controls, and ceiling limitations, upward mobility has basically progressed only on paper. NICHD's main efforts concern job restructuring; this is an attempt to redesign positions to provide opportunities for the upward career mobility of NICHD employees toward more meaningful and productive jobs. For example, we are redesigning most of our lower paying wage grade laboratory support jobs into positions that will gradually involve more responsible duties and define ladders leading into biological laboratory technician career fields under the more flexible and generally higher paying general schedule categories.

As far as most individuals are concerned, the availability of training probably represents the Institute's most significant long-term commitment. More than 150 employees have participated in some type of educational activity within the past twelve months. Twenty-one people are presently enrolled in the NIH Upward Mobility College which gives employees the chance to obtain a bachelor's degree in four years while retaining their full-time employment.

Budget

In the previous year, fiscal year 1971, the Institute experienced an 18% increase in the dollar amount available for funding all types of research and training grants and contracts, and a 2% increase in the number of projects funded. This year Institute funds available for funding all types of research and training grants and contracts and research contracts amount to $94.6 million, increasing 24% ($18.3 million) from the 1971 portfolio, and an increase of 15% in number of projects funded.

Even with these increased funds, over 575 approved research grant applications, representing an estimated dollar value of over $22 million, could not be funded.

While the funds remained constant for training grants and fellowships, significant increases were experienced in research grants and research contract funding. For research grants this represented an increase of approximately 16% in the number of projects funded and approximately 30% in the amount of funds. For research contracts this represented an increase of approximately 29% in the number of projects funded and approximately 30% in the amount of funds. The Center for Population Research accounted for 210 research contracts and reimbursable agreements in the amount of $15.8 million, the Aging program accounted for 29 contracts in the amount of $0.9 million and the Child Health program accounted for 45 contracts in the amount of $4.2 million. The table which follows points up this situation.

Type of Award	No. of Projects	+ or - over 1971	Amount of Dollars (millions)	+ or - over 1971
Research Grants				
Regular Program	867	+119	$54.5	+12.2
Special Programs:				
Mental Retardation Research Centers	12	--	5.6	+ 1.7
General Research Support Grants	--	--	4.0	+ 0.1
Training Grants	120	+ 8	10.0	--
Fellowships	228	+ 8	3.6	- 0.2
Research Contracts	284	+ 63	20.9	+ 4.8

The funding level for the Institute's intramural research program is $8.4 million in FY 1972, an increase of approximately 25% over the 1971 funding level. The balance of the Institute's funds are distributed as follows: biometry and epidemiology - $0.8 million, program management - $4.4 million and payment to the NIH management fund - $4.3 million.

Equal Employment

The EEO Committee is to be commended for its immediate development of a structure and organizational framework, its coordination in working as a team and its problem-solving methods in developing an Affirmative Action Plan. This was done with only a half-time person serving as coordinator of the EEO Committee. The full-time coordinator, Mr. Ted Blakeney was brought aboard this year and through informal interviews became cognizant of dissatisfaction among minorities in EEO accomplishments. A great deal of concern was expressed, both by minorities and by the EEO committee, about the failure to meet raised expectations.

It was pointed out that the EEO Conference had raised minority hopes from a very low level, but that relatively little progress has dashed these hopes once more. Following the recent EEO one-half day sessions the hopes were reluctantly but significantly raised again.

Overall, it may be said that the NICHD EEO effort thus far has achieved a number of goals, such as establishment of:

--a full-time coordinator position
--two training positions in the Office of the Director with extensive efforts being made to obtain minority NICHD candidates
--a Black Advisory Group
--a Task Force on Women
--an employee newsletter

It seems that the NICHD has made a good start in developing an EEO program which is helpful to all employees and which has incorporated, rather than set up as opposing forces, the concerns of minorities and women.

Aging Institute

Considerable pressure has developed within the 92nd Congress for a greatly strengthened Federal research effort on aging, with greater organizational visibility. This pressure has resulted in the introduction of many pieces of legislation, in both houses of Congress, proposing the establishment of a new National Institute of Aging within the National Institutes of Health. Through April of 1972, seven different bills had been introduced to effect the creation of a new institute, while six others were designed to create an Aging Research Commission to conduct a comprehensive and intensive study of the human aging process. In addition, several Joint Congressional Resolutions were offered in support of research on aging and proposing the establishment of a joint Congressional Committee on Aging. The general intent of these initiatives was to assure increased funding and visibility for research on the human aging process.

In an attempt to both entertain Congressional pressure for an enlarged and highly visible research effort, and to stem what was felt to be an undesirable proliferation of research institutes, NICHD prepared a proposal for the development of a Center on Aging Research to be located within NICHD. The

proposal called for the conduct of extramural and collaborative research, the development of a series of aging research centers in educational institutions, and the responsibility for coordinating aging-related research throughout the Federal Government. This plan was forwarded to the Office of the Secretary but was not acted upon.

During hearings on both the House and Senate versions of bills creating a new Institute for Aging, Administration witnesses testified against the establishment of a new institute. Mr. Stephen Kurzman, Assistant Secretary for Legislation, Dr. Robert Q. Marston, Director, NIH, and Dr. Gerald D. LaVeck, Director, NICHD, contended that creation of a new institute was unwise for four general reasons: 1). pre-conditions necessary for great advances in research do not presently exist in the field of gerontology, and experience has shown that such advances in research are not brought about by organizational changes; 2). the immediate step in strengthening gerontological research should be the training of more research personnel, to increase the number of skilled investigators and generate new ideas and leads for research. There is no assurance that a new institute could accomplish this primary goal; 3). indications are that significantly increased administrative costs would result from the establishment of a new institute, which would have to be met at the expense of other high priority health activities; 4). the separation of aging research from research focusing on other segments of the human life span would inevitably result in complex coordination problems, making some degree of duplication of effort likely.

Administration witnesses similarly opposed the establishment of an independent Commission on Aging, on grounds that consolidation of a wide variety of programs dealing with the aged and gerontological research under such a commission would create intolerable administrative confusion.

Recommendations of the Population Commission

The report of the Commission on Population Growth and the American Future, released in March 1971, presented a number of recommendations relating to the population research effort of the Institute. The report strongly endorsed the efforts to increase population research in the biomedical and social/behavioral areas. In order to further encourage the development of such research, it recommended the establishment of a National Institute of Population Sciences. The NIH position on this recommendation is that such an Institute is unnecessary because research programs based on sound assessments of scientific opportunities and responsive to acknowledged social needs do not suffer from lack of funds regardless of organizational location. The present location of population research in NICHD maintains this program in close proximity to the related fields of child health, human development, and aging.

The Commission also urged that the budget for population research be expanded considerably. It specifically recommended that support for biomedical research leading to the development of new methods of contraception be increased to $150 million by 1975. The Commission's report also suggested that "the support for social and behavioral research in population should be increased over the next several years to a total of $50 million annually;" this was not, however, stated as a formal recommendation. The NIH position is that current scientific opportunities do not appear to justify the expenditure of such large amounts of money in the near future, although research funding will undoubtedly continue to expand from the base that has already been established.

Sudden Infant Death Syndrome

The sudden infant death syndrome (SIDS) is the major cause of death in infancy after the first month of life, claiming the lives of three infants for every thousand born. The Office of the Director has moved in three directions to stimulate the development of new knowledge about this complex and tragic medical problem. It has fostered the close cooperation of Institute staff with the Director of Health Statistics Analysis, National Center for Health Statistics, HSMHA, in the establishment of a separate category for SIDS in the ninth edition of the International Classification of Diseases in order to better ascertain incidence of the syndrome; promoted the development of a research planning workshop in August 1971 aimed at defining new directions in research into the causes of the syndrome; and supported the development of a descriptive study of sleep states with a view to identifying infants at risk of SIDS. The Office of the Director has also obligated funds for support of a survey of current practices of management of victims of the sudden infant death syndrome and their families. This survey involves cooperation with a nationally organized group of parents of SIDS victims. The Office coordinated the development of testimony presented by the Assistant Secretary for Health and Scientific Affairs before the Mondale Subcommittee on Children and Youth. The Director, NICHD, appeared as a co-witness with the Assistant Secretary.

Hearings on the sudden infant death syndrome resulted in the development of a joint resolution "relating to sudden infant death syndrome" (S.J. Res 206 and H.J. Res 1070). The purpose of the joint resolution is "to assure that the maximum resources and effort be concentrated on medical research into sudden infant death syndrome and on the extension of services to families who lose children to the disease." The joint resolution provides for the designation of the search for a cause and prevention of sudden infant death syndrome as a top priority in the intramural, extramural, and training efforts of the National Institute of Child Health and Human Development.

Staff members of the OD have spoken to local parent and student groups regarding the sudden infant death syndrome and the Institute's research efforts in this important area.

Pediatric Clinical Pharmacology

The NICHD has always maintained an interest in developmental pharmacology. At one point it was listed as a separate program; subsequent consolidation has placed most of the support of such research in the Perinatal Biology and Infant Mortality Branch, although some is present in other branches. During November 1971, the NICHD Director was invited to attend a meeting dealing with pediatric clinical pharmacology sponsored by the National Academy of Sciences. In response to requests made at that meeting, the Institute invited a few members of the scientific community to Bethesda in January for a discussion of the problem. Research and training needs in pediatric clinical pharmacology were pointed out and special emphasis was recommended for basic research because future progress depends upon such work. Participants agreed that more training is needed but it was emphasized that this could only be successful if it took place where excellent research was already being conducted. Although some of the invited participants felt that substantial support of pediatric clinical pharmacology should come from the NICHD, staff pointed out that many other components of the NIH are and should be heavily involved. Staff stressed the fact that the NICHD already is supporting a substantial program in developmental pharmacology and intends to broaden this; in addition, the Institute would like to be able to support at least one outstanding training program in pediatric clinical pharmacology.

Office of the Associate Director for Program Planning and Evaluation

The Associate Director for Program Planning and Evaluation and his staff are responsible for developing the information needed for the planning and formulation of the Institute's major goals and objectives and the means for appraising progress toward these objectives.

In fiscal year 1972, the Office of Program Planning and Evaluation prepared several reports on aspects of the research and training programs of the Institute in response to requests from Congressional Committees and for inter-agency coordination purposes. Also, staff of OPPE, in cooperation with the Budget Officer and Executive Officer of the Institute, developed the Program Financial Plan for FY 1973-77 and the Operational Plan for FY 1972.

Efforts by various offices of NICHD to expand research on SIDS were initiated and coordinated by OPPE. Staff developed collaborative research projects and defined research goals and needs. OPPE also cooperated in preparing testimony for the Assistant Secretary for Health which was presented before the Senate Subcommittee on Children and Youth (Senator Walter F. Mondale, Chairman) in January 1971, and in the development of plans for an expanded SIDS research program for fiscal year 1973 and beyond.

In the past century the number of medications administered to pregnant women and to children has multiplied enormously. It is possible that at present the fetus is potentially at greater risk from the well-intentioned use of pharmaceuticals than from the stress of labor and delivery. Staff of OPPE, in collaboration with staff of the Extramural Research Program, are cooperating to develop a developmental pharmacology research program that is

concerned with effects of medications administered during pregnancy and childbirth, and during childhood.

During the year, OPPE staff participated in many coordination efforts and maintained liaison with agencies whose missions are complementary to or supportive of the Institute's objectives.

Office of Public Information

During fiscal year 1972 the Office of Public Information (OPI) was the subject of a management and organizational study by the Institute which considered various plans of operation. Late in the fiscal year Ms. Patricia Gabbett became the Public Information Officer. At the same time, the Scientific Publications Section was transferred to OPI from the Scientific Information and Publications Branch, and that branch was abolished. This action consolidated all Institute publication activities under the OPI, which is responsible for the preparation, editing, printing, storage, and distribution of NICHD publications, as well as for all other public information activities. The Gerontology Research Center continues to have its own public information officer in Baltimore.

Several new publications were produced or co-produced by OPI, including the pamphlet, "The Birth Rate and Population Growth in the United States," which won an award for design in the annual Society of Federal Artists and Designers competition.

Congressional and Departmental reports were numerous and became a major task of the OPI this year. Among these were highlights of research progress and the Five-Year Plan for Population Research, with its subsequent Progress Report.

Many TV and radio interviews of Institute staff were arranged by the OPI including an interview about population on the nationwide TV show, "Today."

Press interest in NICHD programs was evidenced in a large turnout for a press briefing on mammalian reproduction and for scientific meetings for which OPI staff managed press rooms. Inquiries from a variety of local, national, and foreign media and organizations were responded to.

The new Public Information Officer is working with her staff and other Institute offices to enunciate and carry through a program of public information responsive to the diverse and changing needs of the Institute.

Office of the Associate Director for Epidemiology and Biometry

This office with its constituent Branches, one for Epidemiology and the other for Biometry, was established in December 1971. The Office of the Associate Director now assumes the responsibility for directing and coordinating the operation of the Institute research and consultation programs

in Epidemiology, Statistics, and Biomathematics and for advising the Director and the other Associate Directors on policy with regard to statistical and other quantitative issues.

During the past fiscal year the Epidemiology Branch has expanded its research activities directed towards the problems of prematurity and infant mortality. Projects initiated or receiving increased emphasis during the year are the epidemiology of Sudden Infant Death Syndrome, and prenatal dietary regimens, the latter study showing promise that the incidence of low birthweight babies can be decreased by more than one-half, considerably influencing the infant and mortality rate. The Branch will add an epidemiologist to the staff in July 1972 to initiate some epidemiologic studies in Aging, an area where very little epidemiology has been done in the past.

The Biometry Branch will continue to highlight a research program in health studies and in statistical problems related to the collection and analysis of data obtained from surveys, clinical trials and laboratory experiments.

The Associate Director has continued to serve in various advisory capacities to other Institutes and agencies and was asked by the Director, NIH, to chair an NIH Steering Committee to review and report on statistical operations at the NIH.

In addition, the Associate Director has participated in certain international activities as a participant in conferences and symposia and as a consultant to the World Health Organization.

Office of Associate Director for
Program Services

One of the major impacts on the Office of Program Services during fiscal year 1972 was the transfer of the contracting authority from the Office of Grants and Contracts, Office of the Associate Director for Administration, OD/NIH, to the Office of Program Services' Research and Contracts Management Branch. A new contract section in the Grants and Contracts Branch was established by combining staff already in the Branch plus staff transferred from the Office of Contracts and Grants. The new contract section has a total of 16 persons. It is expected that 284 contracts and reimbursable agreements valued at about $21 million will be processed by the section during this fiscal year. At the same time there has been a large increase in the number of research and research training grants, research fellowships, and research career development awards handled by the Branch. Together with research contracts they total approximately $95,000,000. This represents about 15% increase in the number of projects (198) and about 25% in dollars ($18.7 million) from last year's portfolio. Also of major importance is the fact that the number of NICHD research grant applications submitted for review during the fiscal year has reached an all time high of 1,280 applications in the amount of $72.2 million. However, during the year only about 32% of the number and 25% of the dollar value were funded. The Institute ranked first or second among all the other NIH Institutes and Divisions in each Council meeting during the year in the number of new research applications reviewed.

The Program Statistics and Analysis Branch was reorganized shortly before the beginning of the current fiscal year and under its new responsibilities participated in the publication of the inventory of Population Research and development of a more sophisticated and expanded computer file for use as a data base for Branch studies and other information needs of the Institute.

The Scientific Conference Branch provided major assistance in the planning and arranging for 17 conferences directly sponsored by NICHD. The Branch is also responsible for the committee management functions for the Institute. The Institute currently has eight advisory committees including the National Advisory Child Health and Human Development Council. The Council has expanded its membership to 14. The Branch Chief, as Council Secretary, has been responsible for assisting in implementing the increased Council membership as well as the Action Plan for the Appointment of Women to Advisory Committees.

Center for Population Research

In fiscal year 1972, the Center for Population Research (CPR) completes its third full year as an organizational unit of the Institute. The CPR administers grant and contract programs supporting research and training in the broad spectrum of population research areas encompassed by the biomedical and social sciences.

Preparation this year of the Department's five-year plan and progress report for population research and services has more closely drawn the lines of coordination required among the Center, the Institute, the NIH, and the Department.

The Center has continued to provide high-priority support to three large and complex programs of population research: the development of improved methods of fertility regulation (including the improvement of contraceptive technology and the control of infertility); studies of biologic and genetic implications of contraceptive use; and investigations of the social science aspects of population problems.

The Contraceptive Development Branch has continued and expanded slightly during this fiscal year. Major effort has been given to expansion of the program of product development, both drugs and devices, which should lead most directly to development of new contraceptive agents. In addition, an important contract was awarded late in the fiscal year to establish a biological testing facility to evaluate compounds for contraceptive activity in both the male and the female. This testing facility will permit expansion of the program of screening selected chemicals from various sources to provide uniform testing and direct comparison of antifertility activity among a large number of compounds. Continued activity during this year in the program of device development has been to study techniques for reversible tubal and vas occlusion by means of implanted devices. Present studies conducted in a variety of laboratory animals may lead to branch support of human clinical trials for devices which prove to be safe and effective.

The programs of the Fertility Regulating Methods Evaluation Branch have expanded at an impressive rate during this fiscal year. For the first time, general solicitations of contract proposals by Request for Proposals (RFP) was made in several areas of contraceptive evaluation studies. New areas entered this year include comparative pharmacology of contraceptive steroids; the interaction of contraceptive steroids with essential dietary nutrients; and a program of research on the medical effects of vasectomy. In addition, research on previously initiated topics received continued support and additional contract awards.

The Behavioral Sciences Branch has maintained its efforts to examine the interrelationships among population size, growth and distribution and economic development and other social and psychological factors and to analyze ways in which individuals are motivated to reduce the size of their families. Development has continued in both major divisions of this program: the determinants of fertility and consequences of population growth, structure and distribution. Particular emphasis has been given to development of more specifically defined research gaps that require information in the area of fertility determinants.

The research and training program supported by the Population and Reproduction Grants Branch includes support of fundamental biomedical and social research, a program of population research centers awards, and grants for research training, fellowships, and career development. The number of research grants in biomedical and social research increased from 225 at the end of fiscal year 1971 to over 275 by the completion of fiscal year 1972. Among these are included four new awards for population research centers.

Active development of collaborative research projects with foreign scientists under the PL-480 program has continued during fiscal year 1972. Although the development of joint applications involving foreign investigators and American colleagues and review procedures in the two countries are necessarily time-consuming, the Center for Population Research now supports eight active projects.

The Interagency Committee on Population Research, for which CPR staff serve as Chairman and Executive Secretary, has conducted and published its third inventory of population research supported by Federal agencies and will again prepare an analysis of this research. This analysis identifies gaps in required knowledge with recommendations to meet them and is intended to inform and guide Federal agencies regarding areas of needed population research.

Expanded scientific information activities during 1972 are expected to yield significant results in fiscal year 1973. Particularly, joint efforts with the National Library of Medicine to produce an expanded bibliography will lead to early trial publication of "Population Sciences: Index of Biomedical Research." Also, development of plans and specifications for a CPR monograph series which will report important research studies in both the biomedical and behavioral sciences is expected to lead to several publications in FY 1973. And, negotiation and award of a contract with the Karolinska Institutet, Stockholm, for development of an International Documentation Center in the population biomedical sciences will provide a valuable international resource.

Office of the Scientific Director

Intramural research programs have undergone expansion and reorganization during this fiscal year. The Developmental Immunology Branch has been established with Clinical Director, Dr. John Robbins, as Chief. The Pregnancy Research Branch has become operational under Dr. Ronald Chez, who has been engaged in planning a three-floor obstetrics and nursery unit to be added to the G wing of the Clinical Center in addition to conducting his own research program. The Section on Molecular Genetics of the Laboratory of Biomedical Sciences has been elevated to Branch status, with Dr. Phillip Leder as Chief. The Developmental Biology Branch has been abolished and its personnel and functions have been assigned to the new Laboratory of Molecular Genetics.

In fiscal year 1972 total budgeted positions for intramural research increased from 244 to 267. In addition a total of 85 scientists worked in NICHD laboratories as Guest Workers, Guest Scientists and Visiting Fellows. Another 34 employees in part-time, temporary, WAE, stay-in-school, and work-study categories aided in intramural research programs. Renovation of laboratory facilities in Building 6 has been completed, and several intramural programs have moved into this space. The improved facilities and capability to geographically unite related programs that had been dispersed will facilitate collaboration and scientific progress.

Selected accomplishments of intramural scientists are indicated below.

Office of the Clinical Director

The research activities of the Office of the Clinical Director during this year were concerned with the scientific and applied problems concerning the nature, pathogenesis, and immunoprophylaxis of diseases in humans caused by Haemophilus influenzae type b. Research problems were concerned with the establishment of an animal model, the identification of the source of "natural" immunity, and characterization of the immune response of the capsular polysaccharide of Haemophilus influenzae type b when injected into children and infants. The Clinical Director's office was also concerned with establishing ties between the Society of Pediatric Research and the National Medical Research Council. Completion of a research program designed to devise new techniques for the isolation to permit more thorough characterization of the serum alpha-1 trypsin inhibitors of humans, and an experimental model of the mouse were completed. The Clinical Director has participated with the Center for Disease Control in evaluating the incidence of Pneumocystis Carinii pneumonia in infants and children with immunologic deficiency diseases.

Social and Behavioral Sciences Branch

A time-sampling observational technique has been developed and refined for describing children's experiences in preschool settings. This instrument was used to evaluate the effects on children of different types of preschool programs. Another observational instrument has been developed and used to investigate the effects of early environmental influence on cognitive and

personality development.

Reproduction Research Branch

Sensitive assays have for the first time been developed which specifically measure and discriminate between human chorionic gonadotropin (HCG) and luteinizing hormone (HLH), thus overcoming a major obstacle in reproduction research. These assays are so sensitive that scientists can now detect HCG as early as the 5th day after conception.

Hybrid somatic cell lines have been created by fusing choriocarcinoma cells with human and mouse fibroblasts and new radioimmunoassays have been developed for several steroids, facilitating studies of adrenal and gonadal function.

The first study of plasma concentrations of estradiol, estrone, estriol, and estrone sulfate throughout pregnancy has been completed.

Studies in men seen in the male infertility clinic with a wide variety of testicular disorders support the hypothesis that tubular factors are important for FSH regulation.

A careful analysis of menstrual histories by scientists in the Reproduction Anthropometry Section has demonstrated a correlation between the length of the menstrual cycle and fertility.

Behavioral Biology Branch

Maternally-deprived rhesus monkeys have been studied as a model of the human maternal deprivation syndrome. In addition to the characteristic severe emotional and behavioral disturbance, the monkeys demonstrated EEG abnormalities.

In order to study synthesis of various nerve cell constituents and their axoplasmic transport, reliable ultramicrotechniques have been developed both to introduce substances into single cells and to isolate any proteins synthesized following stimulus.

Hybrid lines of cells have been successfully formed between neuroblastoma cells and human diploid fibroblasts, and studied with regard to morphology, electrical excitability, and chemosensitivity.

Studies of neuronal membrane permeability have demonstrated that salicylates and related nonnarcotic analgesics increase membrane permeability to potassium and decrease chloride permeability, and that there is a good correlation between their effectiveness in altering neuronal membrane permeability and their relative analgesic potency.

Laboratory of Molecular Genetics

Research directed at understanding the processes involved in transmission of genetic information from one generation to another has enabled scientists

in this laboratory to synthesize DNA complementary to the gene for rabbit globin.

Embryonic chick lens fibers are being used as a model system for studying cell differentiation.

Laboratory of Biomedical Sciences

Studies are continuing of placental and fetal enzymes and isozymes; a laboratory animal model of mental retardation has been developed for the first time; a model system using fetal liver explants in culture has been used to study development of hormonal and nutrient control of enzymes involved in glycogen metabolism; subcellular processes involved in the induction of pharmacologically active enzymes by drugs, polycyclic hydrocarbons, and insecticides have been studied; and the solid-phase technique of peptide synthesis in combination with countercurrent distribution has been used to synthesize membrane-active peptides and gonadotropin releasing factor.

Pregnancy Research Branch

A primate model of diabetic pregnancy has been devised and used to study carbohydrate metabolism. Sequential samples of amniotic fluid obtained from baboon and rhesus pregnancies demonstrated changes in phospholipid/sphingomyelin ratios near term similar to those seen in human pregnancies. A new method has been devised to destroy the fetal pituitary *in utero* without interrupting the pregnancy.

Developmental Biology Branch

The personnel and functions of this Branch were transferred to the new Laboratory of Molecular Genetics during this fiscal year.

Children's Diagnostic and Study Branch

A clinical evaluation of hyperactive children on medication demonstrated marked effectiveness of dextroamphetamine and less effectiveness of chlorpromazine in reducing hyperactivity, compared with activity when the children received no drug or a placebo.

Gerontology Research Center

Laboratory of Behavioral Sciences

A number of studies have focused on changes in learning that occur with age. Research has continued on learned control of cardiac arrhythmias. A patient with the Wolff-Parkinson-White syndrome was able to learn to control her cardiac function to such an extent that she could regulate not only her heart rate, but also to either produce or inhibit premature beats.

Clinical Physiology Branch

A reproducible method was developed for controlling carbohydrate metabolism

in human subjects sufficiently precise to permit controlled perturbation of the system as well as systematic alteration in the components. This technique has led to the demonstration that insulin is distributed in 3 body pools. Tests of renal function as determined by 24-hour creatinine clearance in volunteers in the Longitudinal Aging Study have indicated that loss of function accelerates as age advances. The Longitudinal Study volunteers were also used to assess nutritional status in the elderly.

Laboratory of Cellular and Comparative Physiology

Studies of age-related changes in enzyme levels in the rotifer have shown that eight isozymes of acid phosphatase are present. Of these, five remain constant throughout the lifespan, one decreases, and two increase. The activity of the isozyme which changes most with aging shows a marked increase at the time of cessation of reproduction and a continuous increase thereafter as high mortality rates appear. These observations support the hypothesis that aging may be a programmed phenomenon.

Laboratory of Molecular Aging

A chemical model has been developed to test the hypothesis that senescence results from errors in the incorporation of amino acids into proteins caused by mispairing of DNA or RNA nucleotide bases during replication, transcription, or translation. New RNA-DNA hybridization conditions have been established wherein virtually all of a 28s ribosomal RNA is converted to a hybrid structure and is largely resistant to ribonuclease. A procedure for carrying out these RNA-DNA hybridizations with RNA covalently linked to filters has been successfully worked out. This system has potential application to the isolation of single genes in pure form and determining changes during senescence.

Collaborative Guest Scientist Program

In the past year 10 guest scientist programs were in progress at the GRC. Staff included 26 investigators and 32 support personnel. A number of important observations have come from this program. Treatment of cells in tissue culture with hydrocortisone resulted in an increase of 30-40% in the number of cell divisions and an increased lifespan; and deposition of lipofuscin (age pigment) occurs in male accessory sex glands with aging.

Office of the Associate Director for
Extramural Programs

The four Branches within the Extramural Programs of the Institute (Adult Development and Aging, Growth and Development, Mental Retardation, and Perinatal Biology and Infant Mortality) have endeavored to maintain high quality research and training programs during fiscal year 1972 in spite of the fact that increasing numbers of approved applications cannot be funded.

The NICHD Research and Training Committees established some years ago continue to function smoothly. Their major problems have centered around the review of program projects. The complexity and large size of most program

projects inevitably makes their review difficult. As the shortage of funds has become more serious, the Committees are increasingly adopting the practice of cutting out mediocre sub-projects before approving program projects. They are also looking more and more critically for the overall unity of research goal and the clear interdependence of the sub-projects.

Beginning in fiscal year 1972, the initial review of research career development award applications was transferred from the Research and Training Committees to DRG Study Sections. The new system allows an RCDA applicant to be reviewed by the same Study Section which is responsible for his research grant application. Also, the former dual review of fellowship applications was discontinued by the NIH and the full responsibility placed on the Research and Training Committees.

Perinatal Biology and Infant Mortality Branch

The Perinatal Biology and Infant Mortality Branch is responsible for the supervision and administration of approximately 23 percent of the Institute's extramural holdings, totaling about 267 grants and contracts and amounting to over $12.3 million. The Branch is organized into four broad program areas: Pregnancy and Maternal Health, Developmental Biology, Fetal Health and Development, and Infant Survival and Well Being.

Pregnancy and Maternal Health. The Pregnancy and Maternal Health area of the Branch is concerned with normal and abnormal physiologic events and changes associated with the gravida state during the antepartum, intrapartum, and puerperium periods. Advances are being made with regard to the endocrinology of pregnancy; clinical research in steroid biochemistry; infectious processes and pregnancy; and the role of maternal folate deficiency in the genesis of maternal hypertension, abruptio placentae, fetal malformations, and abortions.

Developmental Biology. The Developmental Biology program area is concerned with fundamental research pertinent to the understanding of human perinatal biology. Knowledge of molecular and cellular biology is integrated at increasing levels of developmental organization by the support of research on systems extending from single celled animals to the more biologically complex systems including primates.

Major areas of research currently supported include the genomic regulation of development, the development of the immune system, developmental endocrinology, organization of the cell, environmental impacts on development, pattern formation and morphogenesis. Recent research emphasis on pattern formation and morphogenesis by PBIM grantees has further clarified the nature of cell surface components, the role of microtubules in determination of cell shape, inductive tissue interactions and gene control of development.

Fetal Health and Development. The Fetal Health and Development area is concerned with a wide range of processes and problems relating to fetal growth and development at tissue and organ levels. Research support aims to further our understanding of normal embryonic development and to clarify the causes, mechanisms, prevention, pathophysiology and treatment of specific events

detrimental to the fetus. Emphasis is given to the interrelationship of pharmacological, nutritional, immunological, metabolic and other physiological factors which are important to fetal health and growth from implantation until parturition.

Infant Survival and Well Being. This program area is concerned with basic and clinical studies of the etiology, pathophysiology, therapy, and follow-up of conditions and syndromes associated with the postnatal period up to one year of age, such as asphyxia, respiratory distress, hypoglycemia, hyperbilirubinemia, anemia, erythroblastosis fetalis, sudden infant death syndrome, etc. Of equal importance are the physiological, biochemical, and behavioral aspects of extrauterine adaptation, including respiratory, cardiovascular, thermodynamic, hematologic, and metabolic changes associated with adjustment to the extrauterine environment.

Through the efforts of a grantee, it is now possible to assess prospectively whether fetal lungs are mature enough to withstand extrauterine existence without developing hyaline membrane disease. Because of this, it is now possible to know whether delivery intervention with a given pregnancy is warranted. The therapy of respiratory distress syndrome has been significantly enhanced through the efforts of grantees also, and the increasingly complex problem of hemolytic disease of the newborn may yield to a new treatment regime which may make therapy a simple process. Follow up of mothers and infants who have been considered high risk in the perinatal period is an integral part of program objectives and goals. During the past year, increased emphasis has been placed on expanding program activities associated with the sudden infant death syndrome particularly as related to its cause.

Growth and Development Branch

The Growth and Development Branch supported approximately 31% of the research grants in the NICHD during FY 1972. This proportion has remained constant for the past several years.

Mechanisms of Biological Growth. The goals of this research area continue to be the elucidation of the nature of the growth process and the exploration of factors and processes which control and promote growth. In scope, it extends from basic studies in developmental biology related to growth at the cellular and molecular levels, to physiological and metabolic studies of growth (e.g., endocrinological studies) in higher organisms including humans. One of the new projects initiated during the year will utilize a unique experimental system to study the genetic control of developmental pathways.

Developmental Immunology. The research supported in this area seeks to clarify developmental events throughout the life span which lead to effective host resistance and to specific immune responses. Both animals and humans are utilized as experimental models permitting insight into basic immune mechanisms.

Developmental Pharmacology. It is important that more knowledge be available on the pharmacokinetics of foreign substances and drugs which are most commonly a problem or in frequent use in treating infants and children. One approach

currently being considered is a drug surveillance project in hospitalized pediatric populations. By this means, the incidence of adverse drug reactions in children can be determined.

Nutrition. The nutrition research supported by the Growth and Development Branch retains a multidisciplinary approach. Emphasis continues to be placed on the role of nutrition in physiological and intellectual development, and the important interactions among nutrition, behavior and environment. Studies of the metabolic, cellular and behavioral factors associated with obesity are another area of program interest.

Physical Growth. Physical growth research supported by the Growth and Development Branch encompasses developmental anatomy, cranio-facial growth, skeletal growth and studies in population genetics relating to anatomical development.

Developmental Behavioral Biology. This area within the Growth and Development Branch is concerned with the biological substrate and mechanisms that mediate behavioral processes. Basic studies on the effects of early experience upon the developing brain and behavior are continuing. Findings to date, if replicated on primates, would have substantial relevance to issues of lactation and breast feedings in human populations. Several studies have important implications for the nature of the sensory environment associated with child-rearing practices.

Learning and Cognitive Development. In its present grant holdings, the Branch supports a broadly based, multidisciplinary effort directed towards understanding learning processes. The current focus includes an analysis of the role of the environment at different developmental stages upon the intellectual development of children, including how information gained from concrete experience is processed, abstracted, generalized, and developed into strategies for problem solving. These studies are investigating the assumption that certain learning experiences are more effective or optimal at certain (critical) stages of development than at other stages of development. Additional research programs are being established to determine what specific learning experiences for any given developmental period will be optimal to maximize intellectual growth and human potential.

Human Communication. This research area focuses on the fundamental research problems associated with the human communicative process and the role of this process in the growth and development of humans viewed both as individuals and as members of a society. Special attention is being given to speech and language development as specific aspects of the communicative process, and to the problems associated with the complex process of visual symbolic reception, perception, and association, which may interfere with the normal development of reading skill. During this fiscal year, as in the past, this area has encouraged a multidisciplinary approach to many of these problems, and, when appropriate, an interdisciplinary effort on a single researchable topic.

Personality and Social Development. Personality and social development of children are concerned with the ways children develop from immature, dependent organisms to fully participating members of society. During the past year, investigators have made various new discoveries. One has found that children begin to make judgments as early as eight months of age. Another investigator has shown that an authoritative (in contrast to authoritarian) style of parental authority is best suited to inculcate certain traits of responsibility and independence in children. Still other investigators have shown that the effects of racially integrated classrooms differ dramatically depending on the attitude of the teacher and the social patterns of each classroom.

Adult Development and Aging Branch

In fiscal year 1972 expenditures for the extramural aging program grew an estimated 28.5%, to $8,060,000. The program supports research and training projects on the biological, medical, and behavioral aspects of aging.

Society, Aging, and Health. The control of infectious and nutritional diseases in this century has been a major factor in the striking alterations that have taken place in the size and age structure of the American population. The United States now has a much larger fraction of older persons in its population than it did at the turn of the century, and much of their morbidity and mortality comes from diseases that had relatively small impact on their ancestors.

NICHD is planning a program that will provide a broad conceptual background for persons concerned with the implications of changing population structure for health and other social problems. Currently it is funding two contracts to review, analyze, and evaluate past and current research in specified areas and to make recommendations for future research in those areas. The areas concern (1) mathematical models for predicting changes in population size and structure as a result of alterations in fertility, migration, and mortality rates, (2) estimates of possible future values for fertility, migration, and mortality, and (3) predictions of the impact that changes in size and structure of the population will have on health, needed health research, public health measures, and health services.

Mental Function. One of the major problems of many old persons both from their own standpoint and from that of society is impaired mental function which may progress to the point of dementia and completely incapacitate those affected. Much remains to be learned in this area. Impairment in cognitive ability probably begins in many persons at least by the middle years of life. In late life a significant number of persons are severely demented. An important conference this year dealt with the relation of primary neuronal disease to aging.

NICHD supports a moderate amount of research on the cognitive defect of age and is supporting studies of the effect of the inhalation of 100 percent oxygen under increased pressure on the mental function of severely demented elderly persons. There is evidence that this may prove of value, but many

more studies need to be done before this therapy can be considered anything other than experimental.

Aging in Women. Last year NICHD supported a conference to lay the foundations for a program to study the causes, manifestations, and treatment of the results of the menopause. At the conference the hormonal changes associated with the menopause, their possible relations to atherosclerosis, osteoporosis, and other diseases, and the possible advantages and disadvantages of replacement therapy were discussed. This year a retrospective study of the effects of replacement therapy, and a study of the hormonal alterations in the post-menopausal period were started.

Aging and Immunology. There is considerable evidence that immunological competence declines with age and that the decrements that occur are important in some of the major manifestations of old age--the diminished resistance of the elderly to infections and very possibly the high incidence of cancer. An increased understanding of the processes involved might well make possible procedures to strengthen immunological competence in old age and thus to improve the health of the elderly. NICHD began the support of several studies in this area in fiscal year 1972.

Cellular Aging. Changes at a cellular level are probably responsible for many of the aging changes that occur at the level of the whole person and are so easily apparent to layman and to physician. Thus the Institute has emphasized research on the cellular processes of aging. NICHD has held a number of meetings of investigators in this field to develop an appropriate program dealing with the phenomenon of limited generation potential of certain mammalian cells. It supports the production of cells suitable for studying it. In the summer of 1972 it supported a course on the use of tissue culture in studies of aging. NICHD now supports a moderately large program of research in this area by the research grant mechanism.

Mental Retardation Branch

The Mental Retardation Branch has primary responsibility for research in the biological, behavioral and social aspects of mental retardation within the context of child health and human development. This responsibility embraces all factors--biological, social and behavioral--that influence subnormal development.

Institute support for research and research training directly related to mental retardation increased sharply during fiscal year 1972 continuing the pattern of rapidly increasing support evidenced during the previous year.

The research supported covers a broad range from almost every branch of the physical, biological, psychological, social and clinical sciences. These investigations are concerned with the etiology, epidemiology, pathophysiology, diagnosis, prevention and amelioration of mental retardation. Program project research grants involving team efforts directed toward solution of major mental retardation problems are strongly represented in the Branch

program of research. These large and complex grants are concerned with both biomedical and behavioral research issues and tend to emphasize research on problems at the interface between disciplines. A major share of Branch research activity is concentrated in the Mental Retardation Research Centers constructed and staffed for the purpose of conducting a major research attack on the problem of mental retardation. Several research topics supported by the Branch are listed below:

Continuing research on PKU provides important information on the efficacy of dietary therapy. In a large scale study of 82 PKU children it was noted that therapy must begin within the first 3 weeks of life to prevent intellectual retardation and can be discontinued after 3-5 years without apparent ill effect.

The concentrated study of inborn errors of metabolism, in addition to the already well known investigations and partial remedies for phenylketonuria and galactosemia, has yielded significant and possibly unexpected results. One investigator, for example, has discovered that kidney transplantation may provide a possible means of controlling Fabry's disease and other inborn errors.

Studies on intrauterine undernutrition and the nutritional status of migrant children lend further support to the role of nutrition in mental development. The analyses of brains from human infants who died at birth and who were small in weight for gestation, indicate significant brain alterations and point to the need for intensive postnatal care of such infants.

The early identification of children with handicapping conditions is an important precursor to effective treatment. One Institute supported investigator has developed a screening instrument to be applied by health aides from poverty areas.

Several types of mental subnormality are associated with chromosomal abnormalities. Significant progress has been made this year in cytogenetic research of relevance to these disorders.

Considerable progress is being made at one of the centers on a large group of genetically determined diseases characterized by the storage and excretion in tissues of mucopolysaccharides. Data indicate that an abnormal polysaccharide pattern can be detected in amniotic fluid. The optimum time for amniocentesis remains to be determined.

Research center investigators at Children's Hospital Medical Center, Boston, have determined that mild brain edema produced by experimental seizures in animals can be prevented by dexamethasone. This may be a lead in the understanding and prevention of some causes of mental retardation in the seizure patient.

Mental Retardation Research Centers. Twelve centers have been created under this program, all of them located on, or closely affiliated with a university. Most have close working relationships with residential or outpatient diagnostic

facilities for the retarded for research and training purposes. All but one of the centers - the University of Wisconsin - have completed construction and occupancy. The research center program, now approximating full operational status, represents the major research thrust of this nation to elucidate the causes and pathogenesis of retardation and to develop methods for its prevention, treatment and amelioration. The complexity of the symptom and its multiple and diverse causes demands the talents of many biological and behavioral scientists in independent and collaborative research activities. The centers, by virtue of their multidepartmental composition are uniquely equipped to implement the interdisciplinary research model.

NICHD Annual Report
July 1, 1971 through June 30, 1972
Office of Public Information

During fiscal year 1972 the Office of Public Information (OPI) was the subject of a management and organizational study by the Institute which considered various plans of operation. Early in the fiscal year the Public Information Officer vacated her post, and the position was not permanently filled until April. At that time, the Institute's information specialist for population, Ms. Patricia Gabbett, became the Public Information Officer. At the same time, the Scientific Publications Section was transferred to OPI from the Scientific Information and Publications Branch, and that branch was abolished. This action consolidated all Institute publication activities under the OPI, which is responsible for the preparation, editing, printing, storage, and distribution of NICHD publications, as well as for all other public information activities. The Gerontology Research Center continues to have its own public information officer in Baltimore.

Publication activities. Several new publications were produced or co-produced by OPI, including "The Birth Rate and Population Growth in the United States" and "Facts About Sudden Infant Death Syndrome" (pamphlets); "Needed Research on the Consequences of Population Change in Developed Societies: Report of a Workshop" and "Research and Training Opportunities for Psychologists in the Population Area" (original papers); "A Five-Year Plan for Population Research and Family Planning Services" and its subsequent "Progress Report" (Departmental reports to Congress). Two documents were revised: "Child Health and Human Development: Research Progress" and "Research and Training Programs of the National Institute of Child Health and Human Development."

OPI editing, publication, and distribution were completed on Development of the Rodent as a Model System of Aging and were in process on another scientific publication, Behavioral Sciences and Medical Education. Editorial assistance was also provided on two books published this year: Abortion Obtained and Denied: Research Approaches and Language by Ear and by Eye: The Relationship Between Speech and Reading.

Publications technically edited in the Scientific Information Centers Branch were: Biggers, J.D. and A.W. Schuetz, eds. Oogenesis. Baltimore University Park Press, 1972; and Wright, S.W., B.F. Crandall, and L. Boyer, eds. Perspectives in Cytogenetics in the Next Decade. Springfield, Illinois, Charles C Thomas, 1972.

Several publications are now being editorially prepared for publication by OPI: Menopause and Aging, Workshop on SIDS, "Annotated Bibliography on SIDS," Respiratory Gas Exchange and Blood Flow in the Placenta, Research Directions Toward the Reduction of Injury in the Young and the Old, Malnutrition and Brain Function: Neurophysiological Method of Assessment, Horizons in Perinatal Research: Implications for Clinical Care, A Workshop on the Mechanisms of the Initiation of Labor, Intravenous Nutrition and High Risk Newborns, and Socialization to Old Age.

Audio-visual activities. OPI staff coordinated and arranged the appearances of four NICHD staff members on the WGMS radio program, "Discussion NIH." Dr. Charles U. Lowe spoke of the intramural research program, Dr. Mortimer B. Lipsett addressed himself to research efforts in reproductive biology, and in a joint appearance, Dr. Eileen Hasselmeyer and Mr. Jehu Hunter discussed the Sudden Infant Death Syndrome. In connection with the emphasis being placed on information and research regarding SIDS, OPI prepared color slides and copy for a TV spot announcement advertising the availability nationwide of the NICHD publication, "Sudden Infant Death Syndrome." TV exposure also included an appearance by Dr. Philip Corfman on the NBC "Today" show to discuss population problems and contraceptives. Dr. Duane Alexander discussed mental retardation on the WTTG (Washington, D.C.) "Panorama" program.

Gerontology Research Center (GRC) staff were featured on two local television shows during the year. Dr. Reuben Andres was a guest on "Opportunity Line" on WBAL and Ms. Marlene Butler, GRC nutritionist, was interviewed on "Woman's Angle" on WMAR. Investigations at the GRC were filmed by the BBC (London), NET (Nippon) Japan Broadcasting, and Bayerischer Rundfunk TV (Munich). Additional GRC inquiries came from KCET-TV (California) WNBC-TV (New York), WMPB-TV (Maryland) and Radio Sweden.

Special Activities. (Exhibits and Scientific Meetings): Although the Institute itself has not engaged in exhibit activities at scientific meetings this year, the expertise of an OPI staff member was called for to represent NIH at its exhibit at the FASEB meetings in Atlantic City. Staff was also available at other scientific meetings to distribute publications and information about the Institute.

(Press Activities): A press briefing on current knowledge and research in Mammalian Reproduction drew a variety of interested reporters to speak with experts gathered from around the world. Similar briefings on other Institute programs are a high priority for OPI in the coming year. The Ambulatory Pediatrics Association, the Society for Pediatric Research, and the American Pediatric Society were afforded the services of OPI staff in operating a press room again this year at the groups' annual meeting. The Gerontological Society has also asked continued OPI operation of its press room at annual meetings. In addition, the GRC information officer managed the physical and mental health section headquarters suite for the 1971 White House Conference on Aging.

(Congressional and Other Reporting): In addition to preparing highlights of research and special reports on research topics for appropriations subcommittees, OPI staff prepared testimony and answers to Congressional committee questions during various hearings on population. Several reports on population research were prepared for the Deputy Assistant Secretary for Population Affairs and the White House. The information specialist for population also helped to plan and prepare an important all-day briefing for an elite body of individuals interested in population. This verbal reporting session was chaired by the Director, NIH, and former Senator Joseph Tydings.

(Annual Reports): An OPI staff member is responsible for the NICHD Annual Report, which has grown from about 100 pages in 1967 to an estimated 1,500

pages in 1972. The number of branch chiefs and associate directors whose reports must be coordinated has more than tripled. The OPI is also responsible for coordination of the Institute's section of the annual Scientific Directory-Bibliography.

(Journal Screening): More than 400 scientific and lay publications are screened each month in order to identify grantee and contractor reports and other articles of interest to the Institute. A listing of all Institute-supported research which is reported in these journals is distributed to Institute program directors and kept on file in the OPI office. This year, 1,315 listings were provided and 300 articles were reproduced for use by the Institute.

(Public, Press, and Congressional Inquiries): Requests for information and publications remain at a high level, with special interest demonstrated in the areas of population, nutrition, and sudden infant death. Among the media and groups requesting information were: Today's Health, JAMA Medical News, Playboy, National Association of Educational Broadcasters, Medical Opinion, Commerce Clearinghouse, Eli Productions, American Educational Research Association, Washington Post, Washington Evening Star, Medical Tribune, Chicago Sun Times, New York Times, Hearst Metrotone News, Drug Research Reports, Pediatric News, OB/GYN News, Medical World News, Wall Street Journal, Ramparts Magazine, Life, Family Circle, True, The National Observer, Chemical and Engineering News, United Press, WNBC-TV, KMOX Radio, AMA-Update, West German TV and Rome Divison of BBC-TV. Verbal presentations were made to groups of graduate students and local classes in response to requests for participation in educational programs.

(Training): Two NIH information aides were trained in NICHD's OPI to prepare for careers in public information upon graduation from college. Both trainees returned to OPI to work under contract after the training period.

Future Plans. The Public Information Officer is studying and consulting with OPI staff on the problems and prospects existing in public information at the present time. During FY 73 goals and responsibilities will be clarified and the function of public information at NICHD expanded.

NICHD Annual Report
July 1, 1971 through June 30, 1972
Office of the Associate Director for Program Planning and Evaluation

The Associate Director for Program Planning and Evaluation and his staff are responsible for developing the information needed for the planning and the formulation of the Institute's major goals and objectives and the means for appraising progress toward these objectives.

In performing these functions, the Office: (1) sponsors and conducts studies on the nature of the emerging problems and needs within the broad field of child health and human development; (2) stimulates and provides guidance for the analysis of the status, character, and extent of scientific activity in problem areas and the resources needed for further Institute efforts; (3) coordinates activities leading to the selection of Institute objectives and the development of program plans; (4) prepares and maintains a comprehensive multi-year program plan utilizing operational planning systems and other analytical techniques; (5) sponsors and conducts reviews of program activities to assess progress toward objectives and to evaluate accomplishments, and (6) maintains an awareness of ongoing Congressional activities, keeps staff informed of legislative developments, and coordinates matters of mutual concern with other interested Governmental and non-Governmental organizations.

Dr. S.W. Greenhouse is Acting Associate Director of the Office of Program Planning and Evaluation of the NICHD. His staff includes an Assistant Director for Clinical Programs, Facilities, and Resources; an Assistant Director for the Behavioral Sciences; an Assistant Director for the Biological Sciences; a Program Analysis Officer; a Program Analyst; a Legislative Reference Assistant; and supporting secretarial staff.

An important continuing activity of the Office is an assessment and review of past NICHD activities in the effort to provide a framework for formulating long-range goals in terms of the Institute's mission and the needs of the '70s and '80s. The basic issue relates to the Institute's orientation, focus, and programmatic directions that will lead to achievements and directions relevant to the world of 1980 and, again, of 1990. The aim is to provide alternative goals and objectives that remain flexible and that are subject to continuing review and modification in terms of the needs of the time.

In Fiscal Year 1972, the Office of Program Planning and Evaluation prepared several reports on aspects of the research and training programs of the Institute in response to requests from Congressional Committees and for inter-agency coordination purposes. There continues to be much interest in how Institute research and training programs pertaining to early child development interface with other Federal efforts in this area. Several pieces of legislation concerned with Federal support of research and services specifically aimed at improving the health status and learning capabilities of children have been introduced in the Congress. All of these bills have some impact on NICHD research activities.

Staff of OPPE, in cooperation with the Budget Officer and Executive Officer of the Institute, developed the Program Financial Plan for FY 1973-77 and the Operational Plan for FY 1972.

The death of infants suddenly from unknown cause accounts for about one-tenth of the total infant mortality in the United States. OPPE staff have initiated cooperative efforts to expand research concerned with the causes and prevention of Sudden Infant Death Syndrome (SIDS). These efforts include staff of The Epidemiology and Biometry Branches, OD, the Perinatal Biology and Infant Mortality (PBIM) Branch, Extramural Programs. This team approach to an important pediatric problem includes development of collaborative research projects and the definition of research goals and needs as well as the coordination of Institute research efforts in this area. Staff of OPPE in cooperation with the PBIM Branch prepared testimony for the Assistant Secretary for Health which was presented before the Senate Subcommittee on Children and Youth (Senator Walter F. Mondale, Chairman) in January, 1971. OPPE staff also assisted in the development of plans for an expanded SIDS research program for fiscal year 1973 and beyond.

In the past century the number of medications administered to pregnant women and to children has multiplied enormously. It is possible that at present the fetus is potentially at greater risk from the well-intentioned use of pharmaceuticals than from the stress of labor and delivery. Staff of OPPE, in collaboration with staff of the Extramural Research Program,are cooperating to develop a developmental pharmacology research program that is concerned with effects of medications administered during pregnancy and childbirth, and during childhood.

The OPPE staff is also involved in contract negotiations with the Social Science Research Council for a study entitled "Genetic Factors in Human Performance," together with the National Institute of Mental Health and the Office of Child Development.

The facilities and resources activity of the OPPE has included the function of coordinator of planning for Building 33, the NICHD Research Facility to be built on the NIH campus. This building, now in final stages of design, will contain approximately 90,000 net square feet. It also includes participation in the development of plans for an intramural clinical research unit in reproductive research and perinatal biology to be activated in the Clinical Center, NIH, via an addition of 15,492 net square feet to be made available through construction of an 8th, 9th and 10th floor on wing G. Target date for occupancy is January 1, 1974. This activity is also included in planning for remodelling of the existing Clinical Center OPD and for a future larger ambulatory care facility.

Interest of OPPE staff continues in Research in Child Development and the Man-Made Environment, and in Consequences of Man's Actual and Potential Manipulation of Reproduction. In regard to the latter, the Institute will publish Dr. James R. Sorenson's comprehensive bibliography on genetic counseling. Staff of OPPE will continue to exploit opportunities to explore new concepts in these areas with a view to defining impact on the direction and progress of Institute programs.

Staff of OPPE participates in coordination efforts, and maintains liaison with agencies whose missions are complementary to or supportive of the Institute's objectives. During the current year these included the Interagency Panel on Early Childhood Research and Development; the Standing Committee on Community Coordinated Child Care (4-C Standing Committee); the Interagency Task Force on Comprehensive Services for School Age Parents (membership on the Steering Committee, the Committee on Research Strategies, and the Committee on Public Awareness); the DHEW Interagency Committee on Planning for Children; the DHEW Intradepartmental Committee for Planning and Implementation of PL 91-695, Lead-Based Paint Poisoning Prevention Act, and DHEW Subcommittee on Research and Statistics Issues (Chairman). An OPPE staff member also serves as NICHD representative at the Organizational Committee Meetings on the Fogarty Center Conferences on Preventive Medicine, as Chairman of the NICHD Safety Committee, and as Chairman of the new NICHD Scientific Publications Steering Committee. The Acting Associate Director continues to represent the Institute at the NIH Advisory Committee for Program Planning and Evaluation.

NICHD Annual Report
July 1, 1971 through June 30, 1972
Office of the Associate Director for
Epidemiology and Biometry

This office with its constituent Branches, one for Epidemiology and the other for Biometry, was established in December 1971. The Office of the Associate Director now assumes the responsibility for directing and coordinating the operation of the Institute research and consultation programs in Epidemiology, Statistics, and Biomathematics and for advising the Director and the other Associate Directors on policy with regard to statistical and other quantitative issues.

During the past fiscal year the Epidemiology Branch has expanded its research activities directed towards the problems of prematurity and infant mortality. Projects initiated or receiving increased emphasis during the year are the epidemiology of Sudden Infant Death Syndrome, and prenatal dietary regimens. A careful review of the records of a pre-natal clinic where special attention is given to good nutrition during pregnancy, avoiding suggestions of gestational weight control and of diuretics and other drugs points to an average increase of three-fourths of a pound in birthweight of this clinic's babies over the birthweight of babies in control clinics drawing from the same high risk population. If complete review and analysis of the data in this study confirms this preliminary finding, additional prenatal nutritional care and education projects will be promoted. It is anticipated that the incidence of low birthweight babies can be decreased by more than one-half and thereby considerably influencing the infant and mortality rate. The Branch will add an epidemiologist to the staff in July 1972 as a Visiting Scientist who will initiate some epidemiologic studies in Aging, an area where very little epidemiology has been done in the past.

As a result of its organizational development from a Biostatistics Section, the Biometry Branch can now formulate and develop its program in a much more efficient and useful manner. The Branch will continue to highlight a research program in health studies and in statistical problems related to the collection and analysis of data obtained from surveys, clinical trials and laboratory experiments. In addition, it will continue and expand its high level statistical consultation activities to scientists in the intramural program and to program directors in the extramural program.

The Associate Director in addition to his administrative and professional duties in directing the Epidemiology and Biometry Programs has during the past year continued to serve as Acting Associate Director of Program Planning and Evaluation. The Associate Director has continued to serve in various advisory capacities to other Institutes and Agencies. He has been Chairman of the FDA Biometry and Epidemiology Advisory Committee and will continue as Chairman for an additional year. He also serves on the following committies:

NCI Biometry and Epidemiology Contract Review Committee

NINDS Epidemiology and Statistics Advisory Committee of the Perinatal Project

HEW Career Service Board for Mathematicians and Statisticians

The Associate Director was also asked by the Director, NIH, to chair an NIH Steering Committee to review and report on statistical operations at the NIH.

In addition, the Associate Director has participated in certain international activities. He was invited to an International Conference on World Population Problems that was concerned with methodological and mathematical issues in research in projection of populations in different countries, in family planning research and in studies of side effects of contraceptives. He helped organize and participated in an international symposium on the considerable statistical problems in studying the effects of environmental pollutants on the health of man. He also served as consultant to the Mental Health Unit of the World Health Organization in the analysis and interpretation of data collected in a major nine nation study on the diagnosis and inter-cultural differences of schizophrenia.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Epidemiology Branch

In December 1971, the Epidemiology Branch was created from the former Epidemiology Section of the Epidemiology and Biometry Branch. This report describes a combination of Section and Branch activities as though the Branch had been in existence since July 1, 1971.

The Branch performs epidemiologic studies in areas related to the mission of the Institute and provides epidemiologic consultation to scientists in both the intramural and extramural programs of the Institute. At the present time Branch research activities focus on two segments of Institute interest: 1) the etiology and prevention of prematurity, and 2) the etiology and prevention of infant mortality. Some Branch projects aim at elucidating the factors which determine birthweight and length of gestation. Other projects are attempts to describe infant mortality trends and identify factors affecting the trends. Still other projects are concerned with the etiology of specific causes of infant mortality: 1) Respiratory Distress Syndrome, the most important single cause of death under 30 days of age, and 2) Sudden Infant Death Syndrome, the most important cause of postneonatal mortality. With the arrival of a new staff epidemiologist in July 1972, the Branch will begin a modest program of epidemiologic research relevant to aging in humans.

Branch research activities received a significant boost by the addition of two persons to the staff in FY 72. Dr. Frank E. Lundin, Jr., a senior epidemiologist who recently received a commendation medal from the U.S.P.H.S. Surgeon General for epidemiologic studies of lung cancer in uranium miners, joined the staff in July 1971. In February 1972, Terrence Corbin, a student at Federal City College, joined the staff as a computer programmer under the auspices of the Cooperative Work-Study Program.

Non-Project Activities

During FY 72 a large portion of staff time was spent in "non-project" activities.

Branch Chief:

1. Served on the NICHD Contract Review Committee.

2. Served on the NICHD Scientific Publications Committee.

3. Continued his teaching activities in the Department of Pediatrics at Georgetown University Hospital.

4. Provided extensive consultation to the Perinatal Biology and Infant Mortality Branch (PBIMB) staff in the development of contract proposals to study 1) the epidemiology of Sudden Infant Death Syndrome (SIDS) in North Carolina, 2) developmental sleep physiology in infants of high and low risk for SIDS, and 3) the effects of maternal methadone addiction on newborns.

5. Provided consultation to staff of the Growth and Development Branch in the development of a contract to study the interactions of nutrition, infection and growth rate in the children of a Guatemalan village.

6. Provided consultation to staff of the Mental Retardation Branch in the development of contracts to study the effects of gestational malnutrition on the birthweight of babies.

7. Consulted with PBIMB staff on plans for possible special SIDS research program to be undertaken by the Institute.

8. Performed a survey of pathologists who had studied SIDS in an effort to establish an epidemiologically suitable definition of SIDS.

9. Recruited a new epidemiologist who will join the Branch staff in July 1972.

10. Traveled to Poland to develop 1) two PL 480 contract proposals to study the effect of induced abortion on the birthweight and gestational age of subsequent offspring, and 2) a PL 480 contract proposal to study the effect of "Post Partum Neurohypophyseal Syndrome" on the birthweight and gestational age of subsequent offspring.

Dr. Lundin:

1. Continued his Staff appointment of Lecturer in the Department of Epidemiology at the Johns Hopkins School of Hygiene and Public Health.

2. Acted as consultant to the University of Louisville study of uterine cancer and cervical cytologic screening.

3. Prepared manuscripts for four papers on cancer among uranium workers.

4. Served as a member of the Committee on the Environment of the Epidemiology Section, American Public Health Association.

Mr. White:

1. Provided the Institute and the Institute EEO Committee with statistics on recruitment, hiring, and promotions.

2. Designed a program which will generate the above-mentioned basic statistics on future occasions.

3. Provided extensive and continuous consultation to members of the Biometry Branch on programming matters.

4. Served as a member of the NICHD EEO Executive Committee.

5. Served as chairman of the Black Advisory Group.

6. Designed a general plotting routine which satisfies nearly all graphing requirements of the Branch.

7. Evaluated the Conversational Computer Statistical System (CCSS) for possible use in the Branch. This system will permit extensive display and analysis of epidemiologic data without the need for specialized programming for each new data set. The Branch began a trial of CCSS after Mr. White arranged through DCRT to access the 360-91 computer at UCLA.

8. Surveyed the statistical literature for tests of differences in spread and, using Monte Carlo procedures, made comparisons among promising new tests. This work will be used for completion of Mr. White's master's degree requirements, and a publication of the Monte Carlo portion is planned.

Several publications resulted from "non-project" activities:

Papers

1. Lundin, F.E., Jr.; Wagoner, J.K.; and Archer, V.E.: Radon Daughter Exposure and Respiratory Cancer, Quantitative and Temporal Aspects. U.S. Dept. of Health, Education and Welfare, Public Health Service, National Institute for Occupational Safety and Health, National Institute of Environmental Health Sciences, Joint Monograph No. 1. Springfield, Va., National Technical Information Service, U.S. Dept. of Commerce, 1971, 176 pp.

2. Archer, V.E.; Lundin, F.E., Jr.; and Wagoner, J.K.: Lung Cancer among Uranium Miners -- 1950 through September 1968. Archives of Environmental Health, in press.

3. Christopherson, W.M.; Mendez, W.M.; Parker, J.E.; Lundin, F.E., Jr.: Carcinoma of the Endometrium: A Study of Changing Rates over a 15 Year Period. Cancer, 27:1005-1008, May 1971.

Talks

1. Archer, V.E.; Wagoner, J.K.; and Lundin, F.E., Jr.: Uranium Mining and Cigaret Smoking Effects on Man. Second Skytop Conference on Respiratory Diseases in Industry, May 18, 1972, Skytop, Pennsylvania.

2. Archer, V.E.; Wagoner, J.K.; and Lundin, F.E., Jr.: Cancer Mortality among Uranium Mill Workers. American Industrial Hygiene Conference, May 14-19, 1972, San Francisco, California.

3. White, Nathaniel B., Jr.: Bivariate Logistic Distributions. George Washington University, Washington, D.C., April 1972.

Project Activities

Serial No. EB-1(c)
1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Role of Maternal and Infant Coagulation Systems in Determining Morbidity among Newborns.

Previous Serial Number: none

Principal Investigator: Charles R. Stark, M.D.

Other Investigators: David Abramson, M.D., N. Vildan Erkan, M.D.

Cooperating Units: 1. Department of Pediatrics
Georgetown University School of Medicine
Washington, D.C.

2. Columbia Hospital for Women
Washington, D.C.

Man Years:
Total: 3 weeks
Professional: 3 weeks
Others: none

Project Description:

Objectives

Primary 1) To determine whether Disseminated Intravascular Coagulation (DIC) occurs in infants with Respiratory Distress Syndrome (RDS).

2) To determine the extent of DIC in premature infants with and without RDS.

3) To determine the extent of DIC in premature infants who succumb to and survive RDS.

Secondary 1) To establish norms for coagulation factors and fibrinolytic activity in premature infants by age in hours.

2) To determine the effects of pitocin assisted delivery on the coagulation system of the newborn.

3) To determine the effects of cesarean section on the coagulation system of the newborn.

Significance to Biomedical Research and Program of the Institute

This study will provide a partial test of the theory which holds that at least one cause of the Respiratory Distress Syndrome is intrapartum disseminated intravascular coagulation. If the study supports the theory or even if it shows nothing more than an association between RDS and DIC, many new approaches to the therapy of RDS will be possible. Because RDS is the principal single cause of neonatal mortality, this research is obviously relevant to biomedical research and the program of the Institute.

Progress and Proposed Course

A complete data set is on magnetic tape and ready for analysis. An anticipated reduction in the principal investigator's "non-project" activities and the availability of the CCSS data analysis system should enable the completion of this project in FY 73.

Serial No. EB-2(c)
1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Season of Birth and Incidence of Respiratory Distress Syndrome.

Previous Serial Number: none

Principal Investigator: Charles R. Stark, M.D.

Other Investigators: Nathaniel B. White, Jr.

Cooperating Units: The Commission on Professional and Hospital Activities (CPHA) in Ann Arbor, Michigan, supplied the basic data under a $8,419 data procurement contract.

Man Years:
Total: 7 weeks
Professional: 7 weeks
Other: none

Project Description:

Objective

To perform a descriptive study of 1) RDS incidence by month of birth, and 2) risk of death from RDS and other causes by month of birth.

Summary

The 2,056,467 birth records that entered the CPHA record system between January 1966, and June 1968, were arrayed in a giant multidimensional table which was forwarded to the Branch on a magnetic tape. Table dimensions were: 1) live or dead at discharge, 2) birthweight $\leq$2,500 gms or $>$2,500 gms, 3) RDS or not RDS, 4) male or female, 5) racial origin, 6) method of bill payment, 7) U.S. census region, and 8) month of birth. This table will provide a basis for determining any association between risk of RDS and risk of death before discharge from the nursery on the one hand and all of the classification variables on the other.

Significance to Biomedical Research and Program of the Institute

A widely held clinical impression holds that RDS occurs more frequently in summer months. If this clinical impression is in fact incorrect, the present study stands a good chance of providing strong evidence against the clinical impression. If RDS occurs seasonally, the study will provide a firm foundation for future studies designed to elucidate the causes of the seasonality. U.S.

vital statistics have long shown distinct seasonal patterns of infant, neonatal, and 1-day mortality rates. The present study will answer the question, "Are these seasonal fluctuations in infant mortality risk independent of seasonal changes in mean birthweight?" Fluctuations in infant morbidity and mortality rates are of vital interest to the Institute.

Progress and Proposed Course

The data is ready for analysis and some initial tables have been created. Serious data analysis will be delayed until more professional time becomes available.

Serial No. EB-3(c)
1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Low Weight for Gestational Age in the U.S.

Previous Serial Number: none

Principal Investigator: Frank E. Lundin, Jr., M.D.

Other Investigators: Charles R. Stark, M.D., Howard J. Hoffman, M.A.

Cooperating Units: National Center for Health Statistics

Man Years:

Total:	24 weeks
Professional:	24 weeks
Others:	none

Objectives

The data for this study make possible the simultaneous classification of births by birth weight and gestational age calculated from the date of last menstrual period as collected by 37 states in 1968. These unique qualities of the data permit us to establish norms for birth weight by gestational age for a general population and to do a descriptive study of low-weight-for-gestational-age babies by race, sex, maternal age, parity, month of birth and geographic area.

Progress

During the year analyses of these data were planned, specific data processing procedures were requested of the Division of Computer Research and Technology, and tabulations were partially analyzed.

Significance to Biomedical Research and the Program of the Institute

Clinically low-weight-for-gestational-age babies have increased morbidity and statistically they have much increased mortality. This descriptive study may provide epidemiologic leads to factors responsible for infant mortality. Related studies planned by the Branch will allow direct estimates of mortality in low-birth-weight-for-gestational-age babies.

Proposed Course

It is anticipated that these analyses will be completed by July 1, 1973.

Serial No. EB-4(c)
1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Relative Efficacy of Two Prenatal Care Regimens

Previous Serial Number: none

Principal Investigator: Frank E. Lundin, Jr., M.D.

Other Investigators: Charles R. Stark, M.D., Charles U. Lowe, M.D.

Cooperating Units: Contra Costa County Medical Services

Man Years:

Total:	27 weeks
Professional:	27 weeks
Other:	none

Project Description:

Objective

To determine whether pregnant women treated with nutrition lectures, no weight control, and no diuretic or appetite suppressant drugs bear healthier babies than women treated with a traditional regimen of weight restriction, diuretics, and low calorie-low salt diets.

Methods Employed

A data procurement contract in the amount of $50,000 is used to obtain coded abstracts of about 5,500 case records of deliveries at Contra Costa County Hospital over a period of 5½ years. Code sheets are forwarded to NICHD for processing and analysis. The Epidemiology Branch will perform analyses to achieve the study objective.

Progress

It appears that the contractor will attain his objective within a one year period; however, due to unavoidable delays in initiation of this work a completion date of August 31, 1972, is projected.

Significance to Biomedical Research and Program of the Institute

It is anticipated that this study will show that avoidance of gestational weight control, avoidance of drugs, and special attention to nutrition will result in a low-birth-weight rate of about 3% and absence of the "toxemia

of pregnancy" syndrome. This result would enable the Institute to plan and execute subsequent studies which would form a basis for recommendations which may ultimately lead to considerable reduction in U.S. infant mortality.

Proposed Course

It is expected that data analysis will be completed during 1972. During FY 73 we plan to collect parallel data on Medi-Cal financed deliveries occurring in hospitals other than Contra Costa County Hospital. These data will be used to rule out selection as an explaination of the results obtained in the FY 72 study.

Serial No. EB-5(c)
1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Risk of Down's Syndrome by Maternal Age and Race

Previous Serial Number: none

Principal Investigator: Nathaniel B. White, Jr.

Other Investigators: Cesar Rudzki, M.D., Charles R. Stark, M.D.

Cooperating Units: Michigan State Department of Health, Lansing, Michigan

Man Years:

Total:	11 weeks
Professional:	11 weeks
Other:	none

Project Description:

Objective

To determine whether the maternal-age effect on risk of Down's Syndrome varies by racial classification.

Summary

Race and maternal-age specific Down's Syndrome rates were computed for a data set comprised of all Michigan Down's Syndrome births for 1950-1967 in the numerators and all Michigan livebirths in the denominators. The result showed racial differences. A manuscript has been submitted for publication.

Significance to Biomedical Research and the Program of the Institute

Does the maternal-age effect on Down's Syndrome differ for whites and nonwhites? This has been and still is a moot question. The present study, however, offers the most authoritative answer. This project is directly relevant to the Institute mental retardation program.

Serial No. EB-6(c)
1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Incidence of Sudden Infant Death Syndrome in the United States

Previous Serial Number: none

Principal Investigator: Charles R. Stark, M.D.

Other Investigators: none

Cooperating Units: National Center for Health Statistics
Rockville, Maryland, and
various state Departments of Health

Man Years:

Total:	4 weeks
Professional:	4 weeks
Other:	none

Project Description:

Objective

To determine mortality rates for SIDS in various states by race, urban-rural, and sex classifications.

Summary

NCHS has agreed to ask for the cooperation of various states to be designated by the Branch. Using the methods of Donald Peterson, the Branch will perform a descriptive study with the objective as stated above. Although we fully realize the limitations of the Peterson Death certificate method, the resulting nationwide overview of SIDS mortality is considered sufficiently valid for current Institute needs and worth the minimal effort required.

Significance to Biomedical Research and the Program of the Institute

This study would provide a better estimate of the national importance of the SIDS problem than any currently available. The Institute needs this information to plan SIDS research programs.

Progress and Proposed Course

In July of 1972, NCHS will petition for state cooperation. Completion of the data acquisition is projected for January 1973, and if the new Branch

Epidemiologist begins work in July 1972, the data analysis should be completed by the spring of 1973.

Serial No. EB-7(c)
1. Epidemiology Branch
2. --
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Independent Effects of Birthweight and Gestational Age on Risk of Mortality in the First Year of Life, New York City, 1964-1967.

Previous Serial Number: none

Principal Investigator: Charles R. Stark, M.D.

Other Investigators: Samuel W. Greenhouse, Ph.D., Frank E. Lundin, Jr., M.D.

Cooperating Units: New York City Department of Health supplied the data set at no cost.

Man Years:

Total:	4 weeks
Professional:	4 weeks
Other:	none

Project Description:

Objective

As stated in the title

Summary

This data set is large enough to permit us to use statistical means to separate the independent birthweight and gestational-age effects on risk of infant mortality.

Significance to Biomedical Research and the Program of the Institute

At various recent times both birthweight and gestational age have been declared "the most important single determinant of infant mortality risk." To date, however, no comprehensive epidemiologic study designed to dissect out the independent birthweight and gestational-age effects has been undertaken. To pursue a rational course in perinatal research, the Institute needs this information.

Progress and Proposed Course

The data set has been acquired and general plans for analysis have been discussed. Real progress cannot occur until other projects are finished.

CONTRACT RESEARCH

Project Titles

I. Development of sleep and cardiopulmonary regulation within sleep: clinical studies of a functional mechanism for risk of sudden infant death.

II. Analysis of state integration and related cardiopulmonary functions in infants at high and low risk for the sudden infant death syndrome.

Contractors

I. University of Southern California.

II. University of California at Los Angeles.

Money Allocated

I. $99,050

II. $101,285

Project Officers

Charles R. Stark, M.D.
Eileen Hasselmeyer, Ph.D.

Objective

To describe the early maturation of sleep "states" and cardiopulmonary function during the various "states" in groups of newborns at high and low risk of Sudden Infant Death Syndrome (SIDS).

Progress

This is a contract to be initiated at the end of FY 72.

Significance to Biomedical Research and the Program of the Institute

At the present time the etiology of SIDS is quite unknown and considered new approaches must be attempted. This project is based on the knowledge that a very high proportion of SIDS events occur during periods of sleep. The working hypothesis is that extreme lability in the autonomic regulation of cardiopulmonary function during REM (active, rapid eye movement) sleep contributes a causal element common to nearly all SIDS events. To make real progress in elucidating the etiology of SIDS, the Institute must open up promising new avenues of research. It is hoped that this project will do so.

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Biometry Branch

In December 1971 the Biometry Section became a Branch. This organizational change followed continually increasing demands placed upon the unit for support of statistical activities within the Institute. This new Branch has two main functions: Consultation and research. The consultation is a service to both the Intramural and Extramural Programs. As will be seen in the list of such consultations, they range from critiques of proposed contracts to analysis of laboratory data. The research activities vary from studies on vital statistics to the development of new statistical methods.

To meet the needs of the Institute, the Branch is increasingly finding it useful to collect, maintain and analyze data files. An example is a registry of amniocentesis as performed in several hospitals to determine whether that diagnostic procedure carries any associated risks. Another is a study of the outcome of pregnancy in women who previously used steroid contraceptives. Third, toward the end of the year negotiation was nearly completed on a contract to continually monitor a large series of births for the purpose of detecting as early as possible epidemics of defects, particularly those that might be related to new environmental agents.

The Branch became especially interested in statistical methods for conducting and analyzing case-control studies. Two papers were submitted for publication. One is particularly provocative, and it is hoped this will stimulate a fresh approach to the analysis of case-control studies.

Activities of the Branch

A. Major Research Activities - Health Studies

1. The Branch is serving as a data center for a collaborative study on the risks associated with amniocentesis, a surgical procedure used in early pregnancy.

2. Analysis is nearly complete on a year's matched births and infant deaths for the United States. The primary purpose of the analysis is to examine the tendency of sudden infant deaths to show a large genetic component.

3. In collaboration with the Epidemiology Branch, a study is underway of whether women who use steroid contraceptives have any ill effects in their future pregnancies.

B. Major Research Activities - Statistics

1. An investigation of the validity of estimates of relative risk in collaboration with Dr. Greenhouse. This research into the way in which covariables must be adjusted for was completed and submitted for publication.

2. A multivariate risk function for case-control studies, in collaboration with Dr. Greenhouse. Completed and submitted for publication.

3. Procedure for analyzing nonstationary biological time series, submitted for publication.

4. Power families: a problem arising from a commonly used transformation. This research led to a publication.

C. Other Significant Activities

The staff is now receiving a very large number of requests from other parts of NIH, of HEW and other areas of government for statistical assistance. Major such consultations follow:

1. Staff has been invited to participate in scientific reviews of contract programs in the Eye, Arthritis & Metabolic Diseases, and Neurology Institute. The same service was provided to AID and EPA.

2. A course in statistical methods in clinical trials of contraceptive agents was given for the WHO, in Teheran, Iran.

3. A member of the staff is serving on advisory groups to the Heart and Lung Institute in its study of arthritis-thromboembolism associations, and to the Drug Monitoring Study directed by Herschel Jick, in Massachusetts.

Consultations

Members of the Branch spent a considerable amount of their time providing consultation for other staff of the Institute. Two are active almost full time with the Gerontology Research Center in Baltimore. One is almost full time with the Center for Population Research. Another is nearly full time on a study for the Intramural program. In addition a very large number of requests are received by Extramural staff, by the Epidemiology Branch, and by Intramural laboratory investigators. The complete list of such consultations would be too long for this Annual Report. A selection of some of the more significant onesfollows:

1. Amnon Abramowitz, NICHD, Regression analysis & analysis of covariance for enzyme levels versus day for saline controls, PCP and PCP plus phenylalanine treatments.

2. Arnold Anderson, NICHD, Two sample problem t-tests and Wilcoxon procedures for activity (mobility) measures versus emotionality (pellets) measures. Experimental treatments were saline controls, phenylalanine & PCP.

3. Dave Alling, Dupont Gerry, David Dale, NIAID, Time series analysis of white blood cell counts (neutrophils, monocytes, lymphocytes, eosinophils, reticulocytes), platelets and haematocrit counts for data collected over an 18 hour span with blood samples every 20 minutes for humans. Similar data, with samples taken daily for two to three months, were analyzed for dogs, some of whom were subject to the disease cyclic neutropenia.

4. Don Gibson, NICHD, Survival function (life table) analysis for a colony of Fishers rats maintained at Charles River Breeding Laboraties, procedure for random selection of animals to be sacrificed, and development of statistical procedures for analyzing necropsy measures. Secondly, analysis of month-specific mortality, fetal wastage, morbidity, etc., for monkey colony maintained at the National Center for Primate Biology, Davis, California.

5. Howard Kulin, NICHD, Development of a statistical method, based on the prediction interval in a regression analysis, for determining threshold effect of suppression on either LH or FSH production, measured in both blood and urine, for varying doses of treatment with an estrogen synthetic hormone.

6. Frank Lundin, Dick Stark, NICHD, Analysis of bivariate tables of gestational age versus birth weight, seasonal patterns of birth weight and relation of birth weight and perinatal mortality to various demographic variables, all part of the Low-weight-for-age study.

7. Bill Orr, Walter Reed Department of Neuropsychiatry, Collaboration on a paper showing forth the methodological considerations of procedures for analyzing nonstationary biological time series, plus results of applying these techniques to twelve experimental subjects: Orr, Hoffman & Frazier, "A 90-Minute Heart Rate Biorhythm: Complex Demodulation and Periodogram Analysis," submitted to I.E.E.E. Transactions on Biomedical Engineering.

8. Floyd Swanson, ODA P, Scatter diagrams, correlation and split-half correlation coefficients, and other techniques were used to establish the validity of substituting the new N.I.H. rating form to the old H.E.W. form. Results were based on samples from nine different worker categories at N.I.H.

9. GRC, Longitudinal Study, (a) planned and is planning computing system for multivariate analyses of longitudinal data; (b) provide extensive variance components analyses of BMR, O_2 consumption, CO_2 production and ventilation volumes to ascertain the magnitudes of the following sources of variability: repeated tests, day to day, repeated visits, among persons; (c) provided graphs and tables to aid in planning the frequency of measurement and the length of study (yrs.) to ascertain longitudinal trends.

10. C. U. Lowe, NICHD, Distributional analyses of nutritional data.

11. C. U. Lowe, NICHD, Multiple regression analyses of factors (pre-pregnancy weight, gestational weight gain) affecting birthweight.

12. Bruce Nisula, NICHD, Experimental design and randomization procedure for clinical trial of drugs used in treating Grave's disease.

13. Reubin Andres, GRC (lab), Development of procedure telling when data should be adjusted for intra-laboratory differences.

14. Ron Chez, NICHD, Analysis of clinical and genetic factors relating to single placentas in Rhesus monkeys.

15. Ken Rosenberg and N. W. Shock, GRC, for planning purposes; Prepared table of sample sizes required to get good estimates of expected mortality in aged mice undergoing air shipment.

16. John Whisnant, NICHD (in progress), Analysis of clinical and genetic factors relating to influenza type 5 antibodies in epiglottitis and meningitis patients.

17. John Schrogie, NICHD, A computer program to determine sample sizes in epidemiologic studies of drugs.

18. Philip Corfman, NICHD, Validity of data from clinical studies of postcoital estrogens.

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Contracts

A member of the Branch serves as primary project officer on five contracts, and as assistant project officer on three studies. The progress of the four contracts in which we hold primary responsibility is given below. The three additional contracts in which the Branch plays a secondary role are: A study of breast cancer and oral contraceptive use, in Yugoslavia; a study of fetal monitoring, in Los Angeles; and a collaboration study of amniocentesis.

In one further contract on the correlates of success in use of intrauterine devices (included in last year's annual report), three manuscripts are currently in preparation, one of which has already been submitted for publication.

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Biometry Branch

Contract and Collaborative Research

Contract Title : A Collaborative Study of Oral Contraception and Cerebrovascular Disease
Contractor : Duke University Medical Center
Money Allocated : $68,750

Objectives: To determine whether the incidence of stroke in young women is increased by use of oral contraceptives, and if so to identify characteristics that are especially related.

Summary: The Neurologic Services of ten university medical centers have identified all young women admitted to their hospitals with definite or suspected cerebrovascular disease of any type. Four of the participating hospitals also identify patients admitted to each of the community hospitals in their metropolitan areas (Miami, St. Louis, Baltimore, and Atlanta). An estimated 200 patients with cerebrovascular disease are submitted for study each year. Clinical data on each of these patients are abstracted from the hospital and physician's records. The frequency of usage of oral contraception in these patients will be compared with that found in two selected control samples: 1) women hospitalized for other diseases and 2) women representing a "healthy" population residing in the same neighborhood as the patients selected for the study. Information regarding pill usage will be obtained from the patient and control subjects by personal interviews carried out in their homes.

Almost all patients and controls have been selected and interviewed in this year. All clinical data have been assembled at Johns Hopkins, where a coding system has been developed. The final year will be spent in data processing, analysis, and development of a report of the study.

All interviewing has been completed. The data have been combined with abstracts of the clinical material. Toward the end of this fiscal year preliminary analyses will have been completed. Within the next several months all analyses will have been completed, and the results of the study will be published.

Significance to Biomedical Research and Program of the Institute: The study is an extention of previous research on thromboembolism and oral contraceptive use in which very few stroke cases were included and is a part of our program to monitor the significant effects on health of currently used contraceptives.

Proposed Course: No further funding planned.

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Biometry Branch

Contract and Collaborative Research

Contract Title : A Comparison of the Medical Effects of Induced Abortion By Two Methods, Curettage and Suction
Contractor : Dr. Franc Novak, Ljubljana, Yugoslavia
Money Allocated : 3 years @ 3,072,675 Dinars(1970-1973)

Objectives: To compare adverse effects to two methods of abortion, suction and curettage. Side effects investigated are bleeding, infection, perforation, subsequent fertility, subsequent ectopic pregnancy, isoimmunization, and cervical incompetence. Study patients to be assigned to these two surgical procedures at random.

Summary: All of the adverse effects mentioned above have been suggested as possible sequelae to abortion. Some have been indicated as being particularly a risk for suction, some for curettage. A considerable shift is occurring in preferance between these two procedures with suction becoming more popular but with advantages of suction never having been resolved. This study provides an especially advantageous situation to do this research for at least three reasons: 1) the large number of abortions performed in Dr. Novak's clinic, 2) the large number of women who have had previous abortions, and 3) the willingness and competence of the staff to conduct a randomized trial.

The design has been successfully implemented; data on over a thousand abortions have already been collected.

Analyses thus far have been of a preliminary nature but useful results are already emerging. For example, it appears as though the amount of blood loss in the two procedures is similar, where it had been suggested that more bleeding would occur with curettage. Further, through a specially designed case control study the risk of ectopic pregnancy does not appear to be increased in women with a history of abortion.

Significance: The safety of abortions as an operative procedure is of apparent public health significance.

Proposed Course: The study will continue through its course of funding.

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Contract and Collaborative Research

Contract Title : Oral Contraceptives and Tumors of the Breast
Contractor : University of California School of Public Health
Money Allocated : $71,552

Objectives: To determine whether the incidence of tumors of the breast is associated with oral contraceptive use.

Summary: The frequency of oral contraceptive use in women with breast cancer and women with benign breast lesions will be compared with that of control groups of women free of cancer and/or breast lesions. Cases diagnosed in a two-year period will be selected.

Three groups of women are being used:

1) Women with breast cancer 49 years of age or younger in whom cancer of the breast has been diagnosed for the first time during the study period. These are patients admitted consecutively to certain designated hospitals in San Francisco Bay Area.

2) Women with benign breast lesions. These are women biopsied or operated for benign lesions of the breast in the same hospitals within a specified period of the cancer cases. One woman with a benign breast lesion is matched with each cancer by age, by race, by religion, and by socio-economic status.

3) Women without evidence of breast lesions or cancer of any site. These women serve as controls. Two controls are chosen for each cancer case, one from surgical and one from the medical service.

The aim is to accumulate at least 400 cases in each of the study groups, women with breast cancer, with benign breast lesions, medical controls and surgical controls.

Basic data necessary for the study are derived from two main sources--review of the hospital record of each subject and a personal interview. The interview seeks information on socio-demographic, menstrual and reproductive and contraceptive variables.

Over 200 cases of malignant breast cancer have been studied, as well as appropriate controls. Preliminary tabulations have been made available to the Institute, so that current surveillance of this important problem can be maintained.

Significance to Biomedical Research and Program of the Institute: This study is an important part of the Institute's program to monitor the side effects that have been proposed as potential sequelae to oral contraceptive use.

Proposed Course: Present plans are to complete data collection in the coming fiscal year, and to then summarize and publish the findings.

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Biometry Branch

Contract and Collaborative Research

Contract Title : A Retrospective Study of the Risks for Cancers of the Breast, Body of the Uterus, Ovary, and Cervix Among Users of Oral Contraceptives
Contractor : Yale University, New Haven, Connecticut
Money Allocated : $93,112

Objectives: To determine whether the incidence of carcinoma of selected sites is associated with oral contraceptive use.

Summary: Cases are being identified of women with breast, uterine, and ovarian cancer admitted to hospitals in the areas in and around Bridgeport, Hartford, and New Haven, Connecticut, and Springfield, Massachusetts, over a two-year period.

Controls are selected as the next patient with the appropriate matching variables admitted to the same hospital as the case with an acute medical or surgical condition or for an elective operation.

Interviews are administered in hospital, whenever possible, inquiring of demographic variables, obstetric history, menstrual history, contraceptive history, and a checklist of diseases.

Analysis will involve an examination of the difference in the proportions of cases and controls exposed to oral contraceptives for different lengths of time, for each site and for specific cell-types at each of the four sites. Relative risks for various lengths of exposure to the oral contraceptive will be calculated. Nearly 200 cases have been interviewed. Preliminary tabulations have been made available to the Institute, so that current surveillance of this important research problem can be maintained.

Significance to Biomedical Research and Program of the Institute: This study is an important part of the Institute's program to monitor the side effects that have been proposed as potential sequelae to oral contraceptive use.

Proposed Course: Present plans are to complete data collection in the coming fiscal year, and to then summarize and publish the findings.

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Biometry Branch

Contract and Collaborative Research

Contract Title : Outcome of Pregnancy in Women Who Have Previously Used Steroid Contraceptives
Contractor : National Naval Medical Center, Bethesda, Maryland
Money Allocated : A reimbursible agreement for $14,535

Objectives: The Institute is interested in determining whether a history of steroid contraceptive use is in any way related to the outcome of subsequent pregnancies. Variables of particular interest are fetal loss, sex ratio, and congenital malformations.

Summary: Information on the course of 50,000 pregnancies is being sought. Procedure, questionnaires, etc. are being developed at the Bethesda Naval Hospital. When the study is operating smoothly, other military hospitls will be included.

Significance to Biomedical Research and Program of the Institute: This study is part of a program to determine the safety of existing contraceptives.

Proposed Course: The study is in its developmental stage and will require up to three years for data collection.

Publications and Talks

Publications

1. Schlesselman, James J.: Power families: A note on the Box and Cox transformation. J. Royal Stat. Soc., B:307-311, 1971.

2. Seigel, D.: The epidemiology of venous thrombosis: Pregnancy, the puerperium and the steroid contraceptive. The Milbank Mem. Fund Quart., L:15-23, January 1972.

3. Seigel, D.: The use of historical data and adverse reaction reporting systems for epidemiologic study. Amer. J. Epid. 94:210-214, September 1971.

4. Seigel, D.: Oral contraceptives: Epidemiologic problems (The chairman's summary of a conference). Amer. J. Epid. 94:190-191, September 1971.

5. Seigel, D.: Oral contraceptives and thromboembolic disorders: Epidemiologic studies. Ann. Intern. Med. 74:258-261, February 1971.

6. Seigel, D.: Letter to the Editor: Thromboembolism and oral contraceptives. Obstet. Gynec. 38:478-479, September 1971.

7. Schrogie, J. and Seigel, D.: Letter to the Editor: Contraception and pulmonary embolism. New Eng. J. Med. 284:984, April 1971.

8. Schrogie, J. Seigel, D. and Corfman, P.: Letter to the Editor: Oral contraceptives and thromboembolic disease. JAMA 220:416-417, April 1972.

9. Seigel, D. and Greenhouse, Samuel W.: Validity in estimating relative risk in case-control studies. Submitted for publication.

10. Seigel, D. and Greenhouse, Samuel W.: Multivariate risk functions in case-control studies. Submitted for publication.

NICHD ANNUAL REPORT
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Center for Population Research
Office of the Director

In Fiscal Year 1972, the Center for Population Research (CPR) completes its third full year as an organizational unit of the Institute. The CPR administers grants and contract programs supporting research and training in the broad spectrum of population research areas encompassed by the biomedical and social sciences. The authorized number of professionals for the Center staff increased from 21 to 24 but some positions remained vacant for most of the year due to difficulties in recruitment.

FY 1972, however, has also been the first year for a number of activities and responsibilities. Enactment of the Family Planning Services and Population Research Act of 1970 (PL 91-572) in December 1970 prescribed requirements for the Department to present to the Congress a five-year plan for research and service activities, and to provide annual progress reports in consecutive years. The required five-year plan was presented to the Congress on October 12, 1971 and was shortly followed by submission of the first year's progress report under the Act to the Congress in January 1972. Preparation of this plan and report has more closely drawn the lines of coordination required among the Center, the Institute, the NIH, and the Department.

Analysis and presentation of the Center's program to meet the goal-oriented requirements for this long-range projection described the four major categories of effort, fundamental research, directed research, product research and development, and institutional support activities pursued by the Center. The following table shows the funds committed to these activities for the past two fiscal years and the organization responsible for their administration.

	(in millions) 71 (actual)	72 (estimate)	
Population and Reproduction Grants Branch			
Fundamental Biomedical Research	7.9	13.1	(20.1)
Fundamental Social Research	2.2	2.9	
Population Centers	0.3	1.5	
Research Training	3.0	2.6	
Contraceptive Development Branch			
Directed Biomedical Research	3.8	4.0	(7.5)
Product Development	2.2	3.5	
Fertility Regulating Methods Evaluation Branch			
Evaluation of Contraceptives in Use	3.1	5.4	(5.4)
Behavioral Sciences Branch			
Directed Social Research	3.9	2.6	(2.6)
Office of the Director			
Scientific Information Services	0	0.3	(0.3)
Staff Support	0.8	1.0	(1.0)
Total	27.2	36.9	(36.9)

Within these categories, the Center has continued to provide high-priority support to three large and complex programs of population research; the development of improved methods of fertility regulation (including the improvement of contraceptive technology and the control of infertility); studies of biologic and genetic implications of contraceptive use; and investigations of the social science aspects of population problems.

The goal for development of an array of effective, safe, inexpensive, and reversible contraceptive methods acceptable to various population groups, has continued to receive emphasis through the efforts of the Contraceptive Development Branch. In May 1971, Dr. Eugenia Rosemberg resigned as Chief of the Contraceptive Development Branch for health reasons. In the absence of a Chief, the successful expansion of the product-oriented program of drug synthesis and testing and continuation of the program dealing with contraceptive devices and drug delivery systems, is directly attributable to the redoubled efforts of the remaining staff members, Drs. Gabriel Bialy, Marvin Karten, and Richard Blye. In November 1971, Dr. Kenneth Savard accepted appointment as Branch Chief effective in early FY 1973.

The initial efforts in contraceptive development by the Center consisted of directed research in a limited number of areas of reproductive biology with special promise of new contraceptive methods. This program has continued and expanded slightly during this fiscal year. Major effort, however, has been given to expansion of the program of product development, both drugs and devices, which should lead most directly to development of new contraceptive agents.

The program in drug development deals with the synthesis of new compounds for fertility control in either females or males. The compounds being synthesized and tested include a variety of chemical structures and several approaches to fertility control. A number of compounds already tested have exhibited contraceptive activity, but whether these will eventually become useful drugs is not yet known. Over 50 new research proposals were reviewed this year leading to award of 10 contracts to implement the wider program in drug synthesis. In addition, an important contract was awarded late in the fiscal year to establish a biological testing facility to evaluate compounds for contraceptive activity in both the male and the female. This testing facility will permit expansion of the program of screening selected chemicals from various sources to provide uniform testing and direct comparison of antifertility activity among a large number of compounds.

Development of contraceptive devices is important in part because the surgical techniques presently available for tubal ligation (female) and vasectomy (male) result in a more or less permanent loss of fertility and reversibility of these procedures requires time consuming surgical operations which are successful less than half of the time. Continued activity during this year in the program of device development has been to study techniques for reversible tubal and vas occlusion by means of implanted devices. Present studies conducted in a variety of laboratory animals may lead to branch support of human clinical trials for devices which prove to be safe and effective.

The programs of the Fertility Regulating Methods Evaluation Branch have expanded at an impressive rate during this fiscal year. For the first time, general solicitations of contract proposals by Request for Proposals (RFP) was made in several areas of contraceptive evaluation studies. New areas entered this year include comparative pharmacology of contraceptive steroids; the interaction of contraceptive steroids with essential dietary nutrients; and, a program of research on the medical effects of vasectomy. In addition, research on previously initiated topics received continued support and additional contract awards.

Initial studies, which emphasize the epidemiological approach to obtaining adequate assessments of risk in use of oral contraceptives, led directly to the expanded group of studies. Contracts for the general surveillance of contraceptive users, clinical studies of carbohydrate and lipid metabolism and of chromosomal breakage in oral contraceptive users, and a continuing study of the physiological effects of intrauterine devices in large animals has yielded much valuable information to date. Surveillance and research studies into the incidence of cancer, cardiovascular effects, genetic effects, and contraceptive effects on offspring have also been expanded during this year. Miss Ruth Crozier was reassigned to this Branch from the Office of the Director, CPR, during this year and has contributed materially to the program expansion effort.

The Behavioral Sciences Branch has maintained its efforts to examine the interrelationships among population size, growth and distribution and economic development and other social and psychological factors and to analyze ways in which individuals are motivated to reduce the size of their families. Development has continued in both major divisions of this program: the determinants of fertility and consequences of population growth structure and distribution. Particular emphasis has been given to development of more specifically defined research gaps that require information in the area of fertility determinants.

The behavioral sciences program of the Center has among its ultimate goals to measure attitudes toward family size and preferences in the use of contraceptive methods; to identify the consequences of various possible future population trends, so that informed choices among them may be made; to show how these trends might be influenced in a pluralistic society, without abridgment of individual freedom; and ultimately, to build a scientific basis for population policy.

Research in the social sciences this year and for the near future include development of studies of the following specific items: preparation for marriage and parenthood; decisions affecting fertility; attitudes toward methods of fertility control; alternatives to childbearing; attitudes determining sexual behavior; fertility and socioeconomic factors; trends in family life; age at marriage; incidence of abortion; population age structure; family planning effects on children; migration; and effects of population on the quality of the environment.

Dr. William Spillane joined the staff of the Behavioral Sciences Branch in an active capacity in August 1971, following two years on Institute-supported research and training at the University of Pittsburgh. Dr. Duff Gillespie

left the Branch to accept appointment with the Office of Economic Opportunity in May 1972.

The research and training program supported by the Population and Reproduction Grants Branch includes support of fundamental biomedical and social research, a program of population research centers awards, and grants for research training, fellowships, and career development. The number of research grants in biomedical and social research increased from 225 at the end of fiscal year 1971 to over 275 by the completion of fiscal year 1972. Among these are included four new awards for population research centers. The five centers awards now in effect at the end of the year are located at the following institutions: 1) Vanderbilt University, Department of Obstetrics and Gynecology (Dr. Burt O'Malley, Director); 2) The Population Council, Biomedical Division, New York City (Dr. Sheldon Segal, Director); 3) University of Texas, Population Research Center (Dr. Harley Browning, Director); 4) University of Wisconsin, Center for Demography and Ecology (Dr. Halliman Winsborough, Director); 5) Harvard University Medical School, Center for Population Research (Dr. Roy Greep, Director). Although available funds increased for research grants in 1972, there was no increase in funds for training activities. It is feared that this prolonged continuation of inadequate funds for training will lead to restriction in the near future of needed expansion of research activities in both the biomedical and social sciences.

Dr. William A. Sadler, Professor and Head, Department of Biology, Texas Southern University, Houston, joined the staff of the Population and Reproduction Grants Branch in May 1972. He is assigned to the staff as a scientist administrator where he will share work and responsibilities in the biomedical area.

Active development of collaborative research projects with foreign scientists under the PL-480 program has continued during fiscal year 1972. Although the development of joint applications involving foreign investigators and American colleagues and review procedures in the two countries are necessarily time-consuming, the Center for Population Research now supports eight active projects. Three others have completed review within the NIH and await external clearances before award. A number of projects in various stages of development in Yugoslavia have received a serious setback during the latter half of this year with the unexpected shortage of PL-480 funds in that country. At this time, prospects for completing developmental work begun in Yugoslavia is in question. If these difficulties are resolved, then continued expansion of the Center's PL-480 program is anticipated with projects in the United Arab Republic, India, Pakistan, Poland, and Yugoslavia in various stages of preliminary development. Coordination of PL-480 activities for the Center are the responsibility of Mrs. Susan Feldman, who joined the Office of the Director of the Center from the NIH Management Intern Program in July 1971.

With the retirement of Dr. Norman Hilmar, former chief of the Program Liaison Branch, and the enactment of the Family Planning Services and Population Research Act of 1970, which specified population coordination and liaison activities were to be located in the Office of the Deputy Assistant Secretary for Population Affairs, the Program Liaison Branch was abolished this year. Mr. Rolf Versteeg was reassigned from the former Branch to the Office of the

Director, CPR, where he has effectively directed the remaining information and liaison activities. Principally, these activities have been in connection with the established Interagency Committee on Population Research, for which Dr. Corfman serves as Chairman, and Mr. Versteeg as Executive Secretary. This group has conducted and published its third inventory of population research supported by Federal agencies and will again prepare an analysis of this research. This analysis identified gaps in required knowledge with recommendations to meet them and is intended to inform and guide Federal agencies regarding areas of needed population research. Work done during this year by a task force of the ICPR has led to expansion of the inventory questionnaire to include information on the scientific discipline of the research project, the organism studied, and the anatomical site involved. Future issues of the inventory and analysis will provide additional summary tables and statistical analyses prepared to make more extensive use of all data collected.

The newly established Secretary's Advisory Committee on Population Affairs met for the first time in November 1971. This Committee, which replaces the function formerly served by the Population Research Advisory Committee for the CPR, meets under the chairmanship of Dr. Louis Hellman. The Committee is divided into two task forces, one dealing with population research and the other with family planning services. Dr. Gordon Douglas is chairman of the Research Task Force and Mr. Versteeg is Executive Secretary. This group has held several meetings during the year for review and analysis of population research programs. A briefing was held by staff of the CPR for the task force following the first full Committee meeting on November 16, 1971. Members of the research task force in addition to Dr. Douglas are: Dr. Judith Davis, Mr. Thomas Dietz, Mrs. JoAnne Gray, Dr. Roy Greep, and Dr. Norman Ryder.

Expanded scientific information activities during 1972 are expected to yield significant results in fiscal year 1973. Particularly, joint efforts with the National Library of Medicine to produce an expanded bibliography will lead to early trial publication of "Population Sciences: Index of Biomedical Research." Also, development of plans and specifications for a CPR monograph series which will report important research studies in both the biomedical and behavioral sciences is expected to lead to several publications in FY 1973. And, negotiation and award of a contract with the Karolinska Institutet, Stockholm, for development of an International Documentation Center in the population biomedical sciences will provide a valuable international resource.

In fiscal year 1972, as in previous years, the CPR supported a number of scientific conferences and workshops to review current knowledge in areas of its concern. The Fertility Regulating Methods Evaluation Branch continued a series of workshops among researchers on studies of hormonal contraceptives and carcinoma of the breast. The Behavioral Sciences Branch held workshops on "Consequences of Population Change in Developed Societies" (June 16-18, 1972), and "The Effects of Population Density on Human Behavior" (October 13-15, 1971), and have one currently planned on "The Causes and Consequences of Fertility Trends in the United States Since World War II." The Center jointly sponsored with the Human Life Foundation of Washington, D.C. a "Research Conference on Natural Family Planning" on January 22-23, 1972, at Airlie House. A publication entitled "Innovative Organization for Population

Research" was released during the year which reported a 1970 conference sponsored by the Center on population research centers. "Oogenesis," published in May 1972, contains the proceedings of a conference held in October 1970, funded by CPR, and organized by Dr. John D. Biggers. Release of two additional books reporting CPR conferences is anticipated shortly: "Mammalian Reproduction" reporting the jointly sponsored CPR-Fogarty Inter-National Center five day international conference on the "Regulation of Mammalian Reproduction" held at NIH in September 1970 and "Human Sterilization" containing the proceedings of a 1969 conference.

Among honors accruing to CPR staff members during this year were appointment of Dr. Philip Corfman, Center Director, to a three year term as member of the Advisory Group to the Expanded Programme of Research, Development and Research Training in Human Reproduction of the World Health Organization by the Director-General in November 1971, and, election of Arthur Campbell, Deputy Director, to the position of President-Elect of the Population Association of America at its annual meeting in Toronto, Canada, in April 1972. Mr. Campbell will preside at the 1973 meeting in New Orleans. Also, Mr. Versteeg, Program Liaison Officer, is the current President of the Association of Population Libraries and Information Centers (APLIC).

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Behavioral Sciences Branch
Center for Population Research

Research relating to population examines the interrelationships between population size, growth and distribution and economic development and other social and psychological factors and analyzes ways in which individuals are motivated to reduce the size of their families. It is, therefore, concerned with a great variety of processes. It takes into account individual, interactional, familial, institutional, and social processes of great complexity involved in decisions relating to family size and the effective implementation of population policies and their consequences. Research in this area has been greatly expanded in line with NICHD's designation as the focal point for DHEW research in population.

Ad hoc advisory panels were established in FY 69 to guide the development of a program of research and to evaluate research proposals which are developed in the behavioral sciences in four areas:

(1) The first of these areas of population research is concerned with the antecedents, processes and consequences of population structure, distribution, and change, and the interrelationships between social and population change. Special importance is attached to the interrelationships between economic factors and population growth, structure and distribution for this and other nations and for particular subgroups of the nation. Of increasing concern is the ecological impact of population growth and of related urban technological and industrial development to guide the formulation of policies with reference to environmental quality. Attention is also being given to migration as a major vehicle of social change.

(2) Major concern focuses on trends in fertility and related variables, such as marriage rates, age at marriage, unintended pregnancies, the incidence of abortion, trends in divorce, and changes in attitudes toward childbearing, family size, methods of fertility control, and other topics, including the failure to prevent unwanted births despite verbal commitment to family planning. Considerable effort is being made to develop a broader data base for more rapid determination of trends and the factors affecting them.

(3) Ultimately the factors affecting trends in fertility will express themselves mainly within the framework of the family, so that a major area of research on population must concentrate on the family, on differences in this institution among various population groups, and on

changes which are occurring in its structure, particularly as they affect fertility. Emphasis must also be placed on sexual behavior, both in and out of marriage, insofar as this bears on fertility. Research is also sought on the process of socialization for marriage and parenthood, including the social pressures exerted by the immediate family, kin networks, peers, and the broader society to have--or refrain from having--children. More needs to be known about the factors influencing decisions that lead to the number and spacing of births, and including perceptions of alternatives to childbearing. Needed, too, is research on the relationship between family structure, childbearing patterns, and child development.

(4) Concern with problems of population growth leads to questions of public policy. Research on all aspects of growth is thus relevant to policy but is also needed specifically on the ways by which population growth or movement are influenced by public policy, and the effects upon population of policies already adopted regarding family planning programs, welfare payments, parity payments, tax exemptions and allowances, direct incentives, etc. As to the relationship with family planning programs, the Center for Population Research is concerned with research on the assumptions under which programs are organized and the evaluation of these programs in terms of those assumptions; operational research is considered the primary responsibility of other agencies.

About one-in-five of the proposals submitted under the contract program in the behavioral and social sciences have been approved by peer review committees and funded. Funding of approved projects totalled $0.7 million in fiscal 1969, doubled to almost $1.6 million in fiscal 1970 and redoubled to approximately $3.5 million in fiscal 1971 and dropped off slightly to $2.9 million in fiscal 1972. Since the initiation of this research program in early 1969 through fiscal year 1971, a total of 52 contracts have been funded. During the current fiscal year, a total of 14 contracts have been approved for funding. Approximately 10 to 12 of these contracts will be funded during the current fiscal year. The remaining contracts will be funded in early fiscal year 1973. Investigators in a wide variety of institutions, mainly universities, but also in governmental and private research organizations, have become involved in the research program. Fields of specialization represented are sociology, psychology, economics, anthropology, medicine, and statistics.

Since the beginning of the Behavioral Sciences Research programs, several studies have been successfully terminated. However, most are still in the active stage. As those now underway and others to be initiated are completed, however, it is hoped that our knowledge of the influences upon the birth rate and completed family size will be greatly expanded.

Much of the discussion about "the population problem" assumes that population plays a large role in many of our other social problems, but to what extent, and in what ways, are not sufficiently well known or agreed upon by competent scholars. Better knowledge concerning the effects of population growth, structure, and distribution are essential if informed and acceptable public policies regarding population are to be formulated and adopted. These studies, dealing with a variety of interrelated factors not easily disentangled, require that a program be structured in long-range terms, and it will be at least several years before results highly useful to policy makers will emerge.

Emphasis here is on projects which were continued (including those for which a final report was received) or funded and initiated in fiscal 1972.

Currently there are 50 funded contracts (including 2 reimbursable agreements with other Federal Agencies), 2 of which are extensions (with additional funds) of projects negotiated in previous years.

Fertility, Abortion, Illegitimacy and Family Planning

Center for Population Research contracts indicate both the concentration of interest in fertility and family planning and the range of problems dealt with.

Under a contract with the University of North Carolina (EA-60)* a study has been undertaken to investigate whether there is a threshold of social and economic development after which family planning action programs will make more rapid progress in Tamil Nadu, India. A Temple University study is designed to assess physicians' current perceptions, understandings, and attitudes toward birth control methods which may be considered as possible abortifacients.

A study on the consequences of illegitimacy, and methodological problems associated with studying the phenomenon, has been instituted at the University of California (EA-12).

An essentially methodological study, arranged under contract with the University of North Carolina (EA-13), seeks to ascertain through computer simulation the effects on fertility of various child-spacing practices in an effort to improve the analysis of current trends in fertility. A University of Pennsylvania (EA-14) project is designed to specify procedures for reducing the cost and complexity of dealing with the 1970 census tapes produced by the Bureau of the Census. This project should have broad applicability across the range of population subjects investigated in the behavioral sciences program of the Center for Population Research but will have specific applicability in permitting a focus on persons rather than on census tracts in local area research on fertility.

*Number of page on which greater detail is given in the summaries of individual projects that follow this general summary.

A contract with DUALabs (EA-66) will develop support techniques to facilitate the wider use of the 1970 Census Public Use Samples (PUS). The project emphasis will be on the handling of very large files by writing programs using COBOL and FORTRAN program to merge different types of records, create smaller work tapes, and provide programs for cross-tabulations and analysis of data. The proposal also includes the writing of users' manuals and provision of classroom instruction in the use of these programs which will be available in the public domain at low cost.

Socio-economic Correlates of Fertility

Six studies deal with socio-economic correlates of fertility in various historical and cultural settings. Analysis of 1967 Survey of Economic Opportunity data dealing with the social and economic variations in marriage and fertility histories has been carried on under a reimbursable agreement with the Bureau of the Census (EA-67). Rand Corporation (EA-16) research is attempting to identify the principal determinants--e.g., income, time of marriage, female labor force participation, and migration characteristics--of family size and current fertility patterns in the United States. The influence of socio-economic factors on fertility should provide the basis for estimating the probable impact on the birth rate of: (1) increased years of schooling, (2) unearned income supplements to the working poor, (3) change in tax deductions for dependents, (4) housing and rent subsidies, and so forth.

A contract with Princeton University (EA-15), a continuation of a study underway for several years, deals with the correlates of fertility reduction in European countries in the last two centuries. The objective of research being undertaken at Western Washington State College (EA-18) is an investigation of social, political, and economic influences bearing on fertility, including rural differentials by size of land holding and abortion and fertility differentials by employment status of women in Poland, and the socio-economic correlates of fertility and birth control in the U.S.S.R.

A contract with the Food Research Institute, Stanford University (EA-19), is investigating the socio-economic correlates of fertility reduction in developing countries. Peasant communities in Indonesia, India, and Mexico and a farming community in the United States will be studied in the field by Columbia University (EA-21) investigators to assess the costs and benefits associated with different family sizes in each community.

Sex Roles and Socio-Psychological Correlates of Fertility

Other studies directly related to fertility approach its analysis from several different standpoints. The 1970 Survey of Fertility, being conducted by Princeton University (EA-22), will analyze fertility behavior and fertility intentions of American women in terms of a variety of social and economic characteristics. In addition, several studies investigate the relation of fertility to marriage and family structure. A contract with Indiana University (EA-23) studies this relationship in terms of the definition of sex roles, emphasizing particularly the effect of employment of women. The University of California at Los Angeles (EA-62) has been awarded a contract to

elucidate the behaviors and motivations of 120 employed career women relating to fertility and family planning and to specify the patterns of temporal ordering of family and work. The Center for Policy Research (EA-47) has undertaken a study of occupational experience as a determinant of fertility among American women. The principal investigator intends to examine an important aspect of the relationship between women's participation in the labor force and their fertility behavior, namely, the characteristics of the occupations they work in. Recently the changing role of women has become more pronounced. Indeed, some persons have termed this change as a "social revolution." There are indications that a large number of women are taking or attempting to take a more active role in the labor market, especially with professional occupations.

The University of Kansas (EA-45) is investigating the dynamics of social norms concerning large and small families--reactions to explicit and implicit social pressures in relation to normatively defined family size ideals. Another contract with the Wright Institute in California is analyzing data that are available on 3,180 Vassar College women during the period 1953 through 1960. This study may reveal many of the current revolutionary themes which may have been latent in the 1950's. For example, women's lib, sexual freedom, decline of the number of children desired, etc., may have been in their embryonic form.

The Center for Policy Research (EA-49) also has underway a research project to examine the possible relationship between ideology and fertility-related behavior. Specifically, the study includes the development of a quantitative model which will examine the relationships between: (1) family background, (2) family ideology (the attitudes and values of the respondent's mother and father), (3) the respondent's ideology, (4) the respondent's career and family role orientations, (5) sexual attitudes, (6) sexual behavior, and (7) preferred family size.

A study, based on a complex model of the relationship between fertility and women's labor force activity being initiated in Research Triangle Institute (EA-24), N.C., suggests that antecedent social and economic variables determine labor force participation and fertility and that labor force participation affects fertility only indirectly. Based on data collected in the Greater Athens Metropolitan Area in 1966-67, a Wayne State University (EA-25) study is examining the antecedent factors relating the employment of women to fertility and birth control to explain how the level of urban Greek fertility can be low in the absence of (a) organized family planning and other governmental programs, and (b) economic industrial development.

Another contract, with the University of Wisconsin (EA-26), analyzes trends in marriage, divorce, remarriage, and their relationships to size of family, using data from the Survey of Economic Opportunity in 1967. Research underway at Bowling Green State University (EA-27) involves a re-interview of mothers interviewed in Toledo in 1963 coupled with a sample of married women in the Toledo SMSA to test for possible associations, within broad religious and socio-economic categories, between selected social-psychological and demographic variables. Under a reimbursable agreement with the Census Bureau (EA-28), a study of the relationship between age at marriage, birth intervals, and total fertility is being made based on a Current Population Survey.

Demographic and Attitudinal Factors Affecting Family Formation

Research underway in Massachusetts by the Worcester Foundation for Experimental Biology (EA-31) is testing attitudinal factors affecting family size. Other research, being undertaken in Louisville, Kentucky, by the Human Resources Research Organization (EA-32), is exploring the factors involved in "moral judgment" with respect to marital status and family size. This study assumes that moral judgments are key factors in social pressures to conform, that single people and childless couples are widely viewed as blameworthy, and that this is deleterious to population control efforts.

Another study, being undertaken by Harvard University (EA-33), looks to the kin network in the United States as the locus of influence for its members with respect to fertility and migration decisions. A University of Kansas (EA-34) study is questioning a group of first-time parents to find factors--"purpose-rational" goal oriented, traditional, or economic--that are affecting their decision-making as to the number and spacing of births.

The University of Missouri (EA-65) is studying the kin network system from the perspective of the elderly (persons 65 years of age or older), with special emphasis on the ways in which migration influences the system. Specifically, this research will: (1) determine the subject's residential proximity to kin; (2) the extent and nature of their interaction with kin; and (3) how these relationships were arrived at. To obtain these goals, 800 persons will be interviewed in Kansas.

Migration, Social Mobility, and Residential Patterns

Fourteen contracts deal with migration. One study being conducted by the Research Institute for the Study of Man (EA-63) will use data from standard and special 1970 and earlier dicennial census tabulations. This study will examine the fertility and its socio-economic correlates of the major Spanish-American population groups in the U.S.: Mexican, Puerto Rican, Cuban, Centra and South American and the descendants of the original (17th and 18th century) Spanish group.

The University of California (EA-61) has under investigation an effort to study the causal structure and decision-making process underlying the migration behavior of the rural poor. This will explore the potential for various public policies which could influence migration from rural and small areas to big city ghettos. The study will test a series of alternative causal models for explaining migration while investigating in depth a model of individual adjustment to economic and social displacement. The principal investigator will conduct a secondary analysis of data previously collected by ABT Associates and funded by OEO. To date, the analysis of these data have been only cursory. Data for 1800 lower socio-economic respondents have been collected.

The University of Texas (EA-51) is studying the demography of the black population. The purpose of this study is to describe and analyze trends in the demographic characteristics of the black population, and compare these with trends in the white population. The basic analytical approach will be a cohort analysis by sex and color.

Battelle Columbia Laboratories (EA-53) is conducting an analysis of migratory responses to employment opportunities. The aim of this research is to clarify the relationship between migration and certain demographic and economic variables and in particular potential employment, i.e., differential migration in response to economic conditions.

A University of Georgia study (EA-35) analyzing the trends and patterns of population change for the total white and non-white populations of all counties in the United States from 1950 to 1960 is concentrating on fertility and migration, as did another study undertaken at Louisiana State University, completed last year.

A Brown University (EA-36) study, based on a probability sample of women residing in Aberdeen, Scotland, who had at least one live birth during the period October 1950-September 1955, is designed to investigate: (1) how spacing of pregnancy and of fertility varies; (2) the life conditions under which women are likely to resort to terminations and sterilizations; and (3) how the size of the family of origin and the position on a traditionalism scale are related to social mobility, present socio-economic status, aspiration for children, and fertility. Upward social mobility, given the economic means and a lack of social barriers, frequently has meant in the United States and Canada a change to different housing in another neighborhood--and perhaps in another city--for, among other purposes, childbearing and childrearing.

The U.S. Bureau of the Census (EA-37), under a reimbursable agreement, will combine data from the March Current Population Survey taken by the Bureau for the years 1966-1970 to study migration during that period by single years of age in order to assess the joint influence of various socio-economic and familial statuses on the probability of making different types of moves during an individual's lifetime if he moved according to present age-specific rates of residential mobility. In another approach to migration research, under a reimbursable agreement with the U.S. Department of Commerce (EA-38), data from the one-percent sample of reports on the Social Security covered workforce are being tabulated to provide a data bank from which other investigators can study mobility among the States, SMSAs, and non-metropolitan areas of the United States. These tabulations will measure in-migration, out-migration, and the non-migrant workforce of each relevant area, cross-classified by age, sex, race, area of origin or of destination, industry of employment, and level of earnings.

A University of Michigan (EA-39) study is analyzing 1970 U.S. census data on tapes for 20-25 SMSAs (Standard Metropolitan Statistical Areas) and comparing these with census materials for four Canadian metropolitan areas to determine: (a) variations in population distribution in different metropolitan areas; (b) the degree of segregation of families in different stages of the life cycle; (c) the degree of residential segregation of different socio-economic groups; (d) the pattern of segregation of housing of different types and ages; and (e) the relation of housing type to socio-economic characteristics of occupants.

Another study deals with the economic costs and benefits of migration to areas which lose and areas which gain from migration. A study being undertaken at

Colorado State University (EA-40) is concerned with the population changes that have occurred during the period 1950-1970--about half of the counties have been losing population--in the Mountain Region stretching from Montana to Arizona and is considering the consequences of those changes to public finances, retail activity, housing utilization, and agricultural activity. The University of Kentucky (EA-41) is conducting an economic analysis of migration from rural Eastern Kentucky to selected urban centers in Kentucky and Ohio to measure the impact of migration in terms of the provision of selected public services by individual donor and recipient local governments and the Federal Government.

In addition, a field survey of four communities in the Philippines also deals with the relation between migration and fertility but seeks to assess the impact of different types of communities of destination on fertility. This study is being done under contract with the University of Colorado (EA-42).

Research Oriented Toward the Possible Development of Population Policies

A Carolina Population Center (EA-43) study is assessing the knowledge and attitudes of three groups (legislators, key leadership groups, and constituent groups) toward population policies in the area of fertility and population growth and expects to generate suggested programs for developing population policies. In a more specific area, a University of Illinois project (is exploring the effect of income on fertility in two different studies: (1) by asking hypothetical questions in a sample survey administered to a regional sample of the U.S. population concerning the effect on personal fertility of government payments for more or fewer children; and (2) by studying the relationship between income and fertility using the 1-in-a-1,000 tape from the 1960 census and demand theory.

The Pennsylvania State University (EA-52) has been awarded a contract to investigate in detail, historically and contemporaneously, the non-metropolitan population changes in the State of Pennsylvania. That State is a large mature area with a highly diversified non-metropolitan sector. The investigators propose to create a data bank of relevant historical-statistical information and to create a typology for the non-metropolitan areas in order to analyze the most important processes shaping change in those areas. Data will be derived primarily from the census but several other sources will be used and field studies will be conducted. The studies will focus on transport, technology, changing labor force roles of females, attitudes toward metropolitan living, and others - which have shaped the non-metropolitan sectors of the population.

TRACOR Inc. (EA-54) has a contract to review research findings on rural-urban migration and develop a complete annotated bibliography.

Future Programs

Several studies already approved but not funded because of a shortage of funds, will be initiated in early fiscal 1973, and additional studies will be developed as increased funds are available. As the Center's Behavioral Sciences contract program continues to expand, emphasis will continue to be placed on studies related to the determinants of fertility. Increasing encouragement will also be given to studies on the effect of population growth and change upon the economy and the environment in an attempt to discover how population growth affects the quality of life. In the development of the program for the near future, an attempt is being made to focus more sharply on critical questions requiring research and a number of specific requests for proposals are being developed to be issued in fiscal 1973.

Each of the studies alluded to above in the general summary is described in greater detail in the summaries of individual projects that follow.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Variations in Cognitive Styles

Contractor: University of North Carolina
Money Allocated: $3,500

Objectives: The purpose is to tape 30 sex information sessions sponsored by Planned Parenthood in Baltimore and to select subjects to be interviewed in order to discover the differences in what instructors think they are saying and what the listeners interpret them to be saying. The tapes and the interviews will be analyzed to abstract dimensions of cognitive style and to establish the congruence and noncongruence of presenter and receiver cognitions. The project aims to describe the types of questions adolescents have, the language they use to communicate about sex, and the types of responses the presenters make to these questions, and to develop an analytic scheme for depicting variations in cognitive style between presenters and receivers and to relate these variations to communication effectiveness. In addition, it seeks to ascertain the sexual behavior of adolescent black youths in a low income urban setting. Its ultimate purpose is to establish guidelines for more effective presentation of sex information.

Significance to Biomedical Research and Program of the Institute: The results of this study will be applicable to sex education programs in this country and abroad and should contribute significantly to the improvement of communication effectiveness in all areas having to do with sex and family planning.

Proposed Course: This contract, begun in 1969, was granted an extension in fiscal 1972 and will be terminated as soon as a final report is received.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A Study of Physicians' Attitudes Toward Abortion

Contractor: Temple University
Money Allocated: $208,053 (1971)

Objectives: The major purpose of this study is to assess physicians' current perceptions, understandings, and attitudes toward birth control methods which may be considered as possible abortifacients. The doctors' attitudes concerning criminal as well as legal (therapeutic) abortions and relevant general attitudes toward fertility intervention and birth control will also be examined. Some 1500 interviews will be given to a national probability sample of M.D.'s and osteopaths whose practice makes the subject of fertility control a reasonable occurrence. The interview schedule will be developed by video and audio taping a series of "In-group" discussions with physicians from four regions of the United States: the Northeast, South, Midwest, and West. The tapes will be analyzed and questions concerning how best to approach the subject of birth control and abortion will be determined. The questionnaire will include the following areas: definitions of pregnancy and of abortion; perception of peers and of the public mood toward abortion; individual perceptions of patients and their circumstances toward abortion; etiology in the correlates of attitudes toward abortion and specific objections to abortion; and the actual behavior of the physician regarding prescriptions of birth control methods and when appropriate, the performance of abortion.

Significance to Biomedical Research and Program of the Institute: Since there is a trend toward the liberalization of abortion statutes and the physician is becoming increasingly involved, it is crucial to determine how the medical profession views abortion. This study accordingly has a great deal of relevance to the Institute's program.

Proposed Course: This project, initiated in 1971, was funded for two years.

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NICHD Annual Report
July 1, 1971 through June 30, 1972

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A Study of Physicians' Attitudes Toward Abortion

Contractor: Temple University
Money Allocated: $208,053 (1971)

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NICHD Annual Report
July 1, 1971 through June 30, 1972

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Illegitimacy - Demographic and Sociological Studies
Contractor: University of California
Money Allocated: $111,076 (1971)

Objectives: The purpose is to develop new information about illegitimate births and expand the methodology available for further study of illegitimacy and related phenomenon. The study, dependent in large part upon available vital and health records, will include the evaluation of California State data on illegitimate births and an analysis of the incidence data for the years 1966 and 1967. The study also includes record linkage cohort studies and an evaluation of interview studies relating to the phenomenon. On the basis of the data to be accumulated, the study will: (1) evaluate State data on illegitimate births; (2) estimate the incidence and distribution of illegitimate births; (3) study the antecedents and consequences of out-of-wedlock pregnancies, as shown by linked vital records; and (4) investigate the feasibility of surveys of attitudes and behaviors in relation to non-marital unions and illegitimate births.

Significance to Biomedical Research and Program of the Institute: This study represents a much-needed basic investigation concerning information which is difficult to get but bears upon a persistent social problem with great significance to the health of both women and children. It will also contribute to the methodology of studying these phenomena.

Proposed Course: The contract initiated in FY 1970, has been renewed with funding through 1972.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: New Estimation Techniques for Demographic Analysis
Contractor: University of North Carolina
Money Allocated: $172,876 (1971)

Objectives: The purpose is to develop sensitive indicators of change in natality patterns. Its specific objectives are to develop and assess appropriate measures for the analysis of "closed" intervals between births and "open" intervals since the most recent birth. Such data are assuming increasing prominence as predictors of natality change and indicators of the effects of family planning programs. The contract will develop new estimators, assess their accuracy, reliability, and sensitivity, and compare various procedures for collecting appropriate data. To evaluate the estimators, an existing microsimulation model will be modified and other special purpose models utilized as necessary. The project should contribute also to more general methods of analyzing current natality data and to the choice of appropriate indices of change.

Under this contract, experiments using augmented simulation models will be devised to (a) simulate reproductive performance, postulating different behavior patterns reflecting different levels and rates of population change, (b) simulate different schemes of sampling in data collection in real populations, (c) calculate from such simulated data the values of the estimators derived above, and (d) assess the behavior of these estimators in response to the conditions of the experiments.

Significance to Biomedical Research and Program of the Institute: The development of more sensitive indicators of current changes in fertility behavior has important implications for both the collection of vital statistics and the evaluation of the effectiveness of public health programs, including those relating to family planning.

Proposed Course: The contract, initiated in FY 1970, was renewed for two years in FY 1971. Completion is expected in early FY 1973.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Preliminary Study of Organization and Procedures Required for Social Science Use of Extended Census Data
Contractor: University of Pennsylvania
Money Allocated: $22,630 (1971)

Objectives: This proposal will specify procedures for reducing the cost and complexity of dealing with tapes produced by the Bureau of the Census in connection with the 1970 census. The cost of a complete data set as issued on tapes by the Bureau of the Census of approximately $100,000, makes this data base largely inaccessible to the great mass of research workers in academic, government, and consulting institutions. This study covers the planning stage of a much larger project to process and distribute 1970 Census Summary Tapes among academic, government and business users who would probably not otherwise have the resources or computer staff and capability to manage the 1970 census tape for purposes of national cross-tabulations or comparisons. This project will produce detailed specifications of: (1) basic methods to be used to reduce the volume and costs of census data tapes; (2) basic methods to be made available for census tape processing in independent institutions; (3) a review of organizational measures and their feasibility, including (a) the formation of consortiums for the production and distribution of usable data tapes and processing methods, (b) feasibility of cost reduction through private support, (c) feasibility and desirability of foundation support, including that of government foundation-type agencies, and (d) recommended organizational forms for a continuing large scale project.

Significance to Biomedical Research and Program of the Institute: Making summary tapes of the 1970 census available is of singular importance to the Institute's program in relation to the population research community.

Proposed Course: This contract is funded for one year into fiscal 1972.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Analysis of the Decline of Fertility in Europe, by Province
Contractor: Office of Population Research, Princeton University
Money Allocated: $21,977

Objectives: The purpose is to analyze the decline in fertility in each of the more than 700 provinces in Europe from the time that marital fertility was essentially constant until the present, or until it reached a minimum and began to rise. Its aim is to determine the circumstances, in terms of the education, religion, occupational structure, residence patterns, etc., of the population, under which fertility declined. This study will make an essential contribution to the social history of Europe and should elaborate considerably upon our understanding of the factors relating to the decline of fertility.

Significance to Biomedical Research and Program of the Institute: Fertility has been designated as an area of the highest priority by the Center for Population Research. This study will furnish information in far greater detail than has previously been available on the social and the economic factors associated with the decline in fertility in many countries of Europe over the past two centuries or so.

Proposed Course: This project, underway for several years, was extended into fiscal 1972.

NICHD Annual Report
July 1, 1971 through June 30, 1972

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: An Investigation of the Determinants of Fertility in the United States

Contractor: The Rand Corporation
Money Allocated: $160,900 (1971)

Objectives: This study attempts to identify the principal social determinants of family size and current fertility patterns in the United States. Using three sets of data (1) Survey of Economic Opportunity 1967, (2) Longitudinal Survey of Family Economics 1967-1970, and (3) a Time Budget Survey taken in 1964, the investigator is concentrating on the effects of such factors as income, time of marriage, female labor force participation, and the timing and frequency of migration upon size of family. The method of analysis consists of multivariate analysis of data from the three surveys.

Significance to Biomedical Research and Program of the Institute: The study of the determinants of fertility and the development of methodologies related to them are one of the major concerns of the program in the Behavioral Sciences of the Center for Population Research. Through the process of multivariate analysis this study will clarify the effect of the various factors already known to affect fertility and will describe with greater precision the behavioral and biological mechanisms affecting fertility and how these differ for various segments of the U.S. population. The study should provide a basis for estimating the probable impact on the birth rate of (1) increased years of schooling, (2) unearned income supplements to the working poor, (3) change in female earnings relative to those of males, (4) change in tax deductions for dependents, and (5) housing and rent subsidies.

Proposed Course: This contract was funded in 1971 for a period of 18 months and should be completed during fiscal 1973.

NICHD Annual Report
July 1, 1971 through June 30, 1972

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Processes of Demographic Development in Imperial Russia and the Soviet Union

Contractor: The Ohio State University
Money Allocated: $107,155 (1971)

Objectives: The purpose is to study the mutual relationships between population growth, economic and social change in Imperial Russia and the Soviet Union. In order to identify the long-term factors that determine and influence demographic changes, the study will explore the determinants of Soviet population growth from a long-run point of view, which also includes the early stages of change during the Imperial Russian period, and to appraise the Soviet efforts to design and implement population policies to influence fertility patterns and other demographic variables. The principal themes of the proposed research will be those concerned with the implications of the developmental process for population growth, with major attention given to t question of fertility.

Significance to Biomedical Research and Program of the Institute: The study of demographic developments during a process of economic development and social change during the 18th, 19th, and 20th centuries in a society which shows both European and Asiatic influences is of the highest relevance in understanding the process of population change in the world at large. The findings will increase knowledge of the factors associated with declining fertility, the use of contraceptives and of abortion, and will provide some insight into the effectiveness of a variety of population policies.

Proposed Course: The original contract, initiated in 1970, was renewed with funding for FY 1971 and FY 1972. Completion is expected in FY 1973.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Human Fertility in Poland and the U.S.S.R.

Contractor: Western Washington State College
Money Allocated: $47,169 (1971)

Objectives: The primary objective is an investigation of social, political, and economic influences bearing on fertility in Poland and the U.S.S.R. and including rural fertility differentials by size of land holding, and abortion and fertility differentials by employment status of women in Poland, and the social and economic correlates of fertility and birth control in the U.S.S.R.

Since the mid 1950's the Soviet Union and the Eastern European countries within its sphere have published more demographic and economic data and have liberalized abortion laws (partly because of limited housing and the need to increase the number and proportion of females in the labor force).

The demographic processes in the Communist countries need to be studied for the light they may shed upon relationships of population to socio-economic-political conditions which are both similar to and different from those in other regions. Fertility differentials between the more developed parts of the RSFSR on the one hand and the five Union Republics of Central Asia and of the Caucasus, and of the autonomous republics on the other hand should throw additional light on basic demographic processes in various cultural contexts. In addition, the effects of sharp and contradictory policy changes need exploring both to assess the effects of such shifts and to avoid similar problems in the U.S.

Significance to Biomedical Research and Program of the Institute: All aspects of the research are program relevant, but particularly the impact of abortion, of urban-rural, religious and other ethnic differentials and, more generally, of "modernization," on fertility trends. Moreover, the investigator is one of a very small number of persons in the U.S. with both the language and technical skills to pursue studies in the Slavic countries.

Proposed Course: This study was initiated in 1970 and will be completed in early FY 1973.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Socio-economic Factors in the Reduction of Natality in the Less Developed Areas

Contractor: Food Research Institute
Stanford University
Money Allocated: $65,698 (1971)

Objectives: The objective is to examine the usefulness of the notion of the demographic transition, or modifications thereof, as a model for fertility reduction in non-Western areas, and to determine the context of socio-economic conditions that appear to be the basis for the onset of a decline in fertility in the underdeveloped or developing countries of the world. Measures of natality and of socio-economic development are being studied in each of the several major regions of the world to determine their accuracy and comparability, and to analyze their interdependence. The study will also seek to provide a possible measuring stick or projective device for estimating the effects of efforts to reduce fertility in the future.

Significance to Biomedical Research and Program of the Institute: This study will provide insight into the process of fertility decline in the developing and underdeveloped regions of the world and the extent to which the socio-economic conditions associated with natality decline in the West are having, or are likely to have, the same effect in the non-Western world, as well as indicating what additional factors may influence fertility decline in these areas.

Proposed Course: This contract, begun in FY 1970, was renewed with funding for FY 1971 and will be continued into FY 1973.

NICHD Annual Report
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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Urbanization, Migration and Fertility in Thailand

Contractor: Brown University
Money Allocated: $92,475 (1971)

Objectives: The purpose is to analyze census data and data from a national longitudinal survey of Thailand to determine the relationship between urbanization, migration, and fertility. The fertility data available from this national survey, cross-tabulated by residence and household type, permit their evaluation in terms of a rural-urban continuum, and their control for age differentials provide the opportunity for comparisons of fertility levels across the urban-rural continuum for differentials by religion, literacy, education, economic status, migration, and marital status.

More specifically, the study will analyze: (1) cross-sectional and trend data on urbanization and on the relationship between fertility and socio-economic variables based on extensive data collected in the 1960 Thai Census and preliminary data from the 1970 Census; (2) rural and urban data from the National Longitudinal Survey of Social, Economic, and Demographic Change in Thailand collected 1968-1970 to ascertain: (a) total migration experience of the population: (b) the role of population and economic pressures on movement away from the village of birth; and (c) the relation between migration and fertility; (3) rural-urban labor force, occupation, and industrial differentials and data on literacy, education, and religion; and (4) data for integrating the material from the rural and urban survey operations.

Significance to Biomedical Research and Program of the Institute: The study will add to available knowledge of rural, urban, and other socio-economic differentials in fertility in an area for which data have previously been non-existent, and for a culture which is quite different from that in the United States. Hence, it will add another perspective to the understanding of influences upon fertility behavior.

Proposed Course: The contract, initiated in 1970, is anticipated to run for a period of three years.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Economic Cost and Value of Children in Four Societies

Contractor: Columbia University
Money Allocated: $215,320 (1971)

Objectives: This research is directly aimed at the assessment of the importance of economic costs of, and returns from, children as possible factors in fertility decisions. The secondary aim will be the development of refinement of research methodology for such studies.

Peasant communities in Indonesia, India and Mexico and a farming community in the United States will be studied using anthropological techniques of intensive observation together with extensive questionnaire surveys. Field investigation and data analysis will be carried out by four research assistants familiar with the particular communities in which they will be doing the research. Intensive observation of domestic and productive activities will be used to assess the costs and benefits associated with different family sizes in each community. Census and fertility surveys of the four populations will be carried out to determine actual fertility and attitudes regarding fertility. Data analysis in the third year will provide comparable data for all societies.

An accurate census will be taken with respect to demographic information, socio-economic status, land holdings, etc. The communities studied will each comprise about 400 households of which a sample will be selected for intense study to include: (a) households in different stages in the developmental cycle, etc.; and (b) the organization and productivity of labor, and the econmic cost of children, both direct and indirect. A third sample will be comprised of all married women of reproductive age or a randomly drawn sample of such women with interviews to be based on the short list of variables for comparative fertility studies from the International Union for the Scientific Study of Population.

Significance to Biomedical Research and Program of the Institute: This study deals with a crucial area of population research that has definite relevance to the Institute's program. Very little information has been available on the ecnomic cost or benefit of children and how these are perceived, even for industrial societies.

Proposed Course: This study, initiated in 1971, is a three-year project expected to be completed in FY 1973 or early FY 1974.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: National Fertility Survey, 1970-1975

Contractor: Princeton University
Money Allocated: $390,871

Objectives: The objective is to survey spproximately 6,500 women representing a national sample of all ever-married women under age 45 in the coterminous U.S. to determine fertility behavior and fertility expectations of American women. The study is a continuation of studies begun as the Growth of American Families Study, 1955, repeated in 1960, and of a similar National Fertility Survey done by the present investigators in 1965. The study will compare fertility behaviors and expectations by age, marital duration, race, education, income and religion, and will investigate the use of various contraceptive methods, and the influence of a broad range of factors on their use. It will include for the first time a sample of women previously married but not currently in the married, husband-present category. Data developed in this study will be augmented to overcome shortcomings observed in the previous studies, e.g., by analyzing fertility by duration of marriage as well as by age.

Significance to Biomedical Research and Program of the Institute: This study will continue a unique series of studies on fertility, fertility expectations and contraceptive practices among women in the U.S., and will provide a base of reference for prospective studies on contraceptive practices and the influences upon the health of women and children. It is an essential part of the public health aspect of the study of contraceptives as well as of attitudes toward family size.

Proposed Course: This contract, initiated in 1970, will be continued into fiscal 1973.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Sex Role, Family Structure, and Fertility Control

Contractor: Indiana University
Money Allocated: $37,120 (1971)

Objectives: The purpose is to study the relationships between definitions and perceptions of male and female roles within the family and achieved and anticipated fertility. The specific concern of the study is with those decisions relating to childbearing and with the antecedent conditions that structure them, with emphasis upon the husband-wife interaction and, particularly, with reference to the alternative sets of roles and rewards which are perceived as valued and open to the female. Emphasis will be placed upon the effect of wife's employment and career orientation upon decisions with respect to the size of family. The contract involves the interviewing of a regional probability sample of 3,000 persons, 15 to 45 years of age, drawn from the East North Central Region of the United States.

Significance to Biomedical Research and Program of the Institute: This contract bears on many aspects of the Institute's concern with contraceptive practice and family planning: age at marriage, decisions affecting fertility, alternatives to childbearing, such as employment of the woman, probability of divorce, and socialization for marriage and parenthood.

Proposed Course: This contract was initiated in FY 1970 at a funding level of $207,260. It was renewed in FY 71 with $37,120 in funds. The progress of the project has been excellent, but unavoidable delays were experienced with the survey. As a result, it has been extended without further funding until February 28, 1973.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Women's Labor Force Participation and Fertility

Contractor: Research Triangle Institute
Money Allocated: $110,664 (1971)

Objectives: This two-phase study is based on a complex causal model of the relationship between women's fertility and their labor force activity. The model suggests that antecedent social and economic variables determine labor force participation and fertility and that labor force participation affects fertility only indirectly. It will not duplicate work underway at the University of Wisconsin.

Phase I entails the secondary analysis of at least two of four previously collected data sets which report on the fertility behavior of American women of childbearing ages: (1) Growth of American Families Survey, 1960; (2) Survey of Work Experience, 1967-70; (3) Family Growth in Metropolitan America Survey, 1957-60; and (4) the Survey of Economic Opportunity, 1966-67. Data from the Survey of Work Experience and Survey of Economic Opportunity will be used to test the hypotheses resulting from the causal model and allow the researcher to formulate a design and instrumentation for the second part of the study. Phase II will develop and administer an interview schedule to a sample of 300 women and a sub-sample of 150 of their husbands. The pilot study will attempt to develop new techniques for determining attitudes relevant to fertility behavior not covered sufficiently in the above studies and will serve as a check for the causal model developed. If these two phases of the study are completed satisfactorily, the principal investigator plans to submit a proposal entailing a national sample.

Significance to Biomedical Research and Program of the Institute: The proposal deals with a very crucial area of population well within the Institute's interests. There has been much written about the relationship between women's labor force participation and its influence on fertility behavior, but there is still much confusion about this relationship. This proposal approaches the problem in a sophisticated, fresh manner, and even if the causal model does not work, the descriptive data amassed will allow researchers to construct new conceptual and methodological approaches.

Proposed Course: This project, funded for a total of two years, is expected to be completed early in FY 1973.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Correlates of Low Fertility in a Developing Country

Contractor: Wayne State University
Money Allocated: $17,200

Objectives: This study will examine the antecedent factors to explain how the level of urban Greek fertility can be low in the absence of (a) organized birth control and other governmental programs, and (b) economic industrial development.

Data already collected in the Greater Athens Metropolitan Area in 1966-67 will be analyzed to study: the relationship of the employment of women to fertility and birth control, examining three aspects of female employment: (a) the degree of compatibility between the working and mother roles, (b) the type of occupation, and (c) the degree of work commitment.

Significance to Biomedical Research and Program of the Institute: The central problem of trying to account for the low birth rate in Greece without much industrialization or family planning programs is of considerable theoretical and possibly practical significance. The project is highly relevant to the Institute's interests in the determinants of fertility.

Proposed Course: It is expected that the work on this contract begun in 1971 will be completed during fiscal 1972.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A Study of Differentials and Trends in Marital Disruption, Remarriage, and the Fertility of Remarriage

Contractor: University of Wisconsin
Money Allocated: $42,178 (1971)

Objectives: The purpose is to examine patterns of marital disruption, remarriage, and the fertility of remarriages in the United States from data obtained in the 1967 Survey of Economic Opportunity, which involved a large sample and covered the national population. The study will be concerned with (a) differentials among socio-economic groups and racial groups, (b) the patterns of divorce and widowhood in relation to age, marriage, duration, color, education, age at marriage, order of marriage, sex, and presence of children, as well as place of residence, and the length of the interval between marriage and the birth of the first child, and with patterns of remarriage in relation to the above; and (c) how these patterns have changed through time. It will also investigate the pattern of fertility of second and subsequent marriages and how differential fertility and change in fertility is influenced by differential marital disruption.

Significance to Biomedical Research and Program of the Institute: This study contributes to the understanding of the role played by marital disruption and remarriage in the practice of contraception and attitudes toward family size.

Proposed Course: This contract, initiated in FY 1970, is expected to be completed during FY 1972.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Social-Psychological Correlates of Urban Fertility

Contractor: Bowling Green State University
Money Allocated: $34,367

Objectives: This research, involving a re-interview of 754 mothers interviewed in Toledo in 1963 and a sample of 3,000 married women in the Toledo SMSA, has several interdependent objectives designed to test for possible associations, within broad religious and socioeconomic categories, between selected social-psychological and demographic variables: (1) to determine by means of longitudinal study the degree to which present family size can be predicted from alienation scores obtained from the sample of mothers in 1963; (2) to determine the extent to which the obtained differentials in fertility by alienation variables in previous research would be accentuated and refined in a community-wide sample that would include mothers who are continuing their childbearing as well as mothers who have successfully terminated their fertility; (3) to identify some of the social and demographic factors associated with differential kinds and degrees of alienation among mothers at given age, parity, and marital duration levels; (4) to determine the extent to which selected husband-wife interaction patterns are associated with differential alienation-fertility relationships; and (5) to derive a conceptual model of differential family planning and fertility behavior by combining stratification, religion, mobility, alienation, family, and information variables.

It will concentrate on attempts to understand better fertility and family planning differentials within social class and religious categories. Alienation variables will be considered as intervening between social structures and fertility, and the primary emphasis will be on specifying the conditions--of education, religion, age, etc.--under which particular patterns of alienation dimensions may be causally related to the dependent variables.

Significance to Biomedical Research and Program of the Institute: A study of the social psychological factors affecting fertility will make an important contribution to our understanding of fertility behavior, the sine qua non of the Institute's program in population research.

Proposed Course: This study was funded for $77,424 in 1971, and extended with additional funds in 1972. It is expected to be completed in FY 1973.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A Study of Differentials and Trends in Marital Disruption, Remarriage, and the Fertility of Remarriage

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Money Allocated: $42,178 (1971)

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Significance to Biomedical Research and Program of the Institute: This study contributes to the understanding of the role played by marital disruption and remarriage in the practice of contraception and attitudes toward family size.

Proposed Course: This contract, initiated in FY 1970, is expected to be completed during FY 1972.

NICHD Annual Report
July 1, 1971 through June 30, 1972

Behavioral Sciences Branch
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It will concentrate on attempts to understand better fertility and family planning differentials within social class and religious categories. Alienation variables will be considered as intervening between social structures and fertility, and the primary emphasis will be on specifying the conditions--of education, religion, age, etc.--under which particular patterns of alienation dimensions may be causally related to the dependent variables.

Significance to Biomedical Research and Program of the Institute: A study of the social psychological factors affecting fertility will make an important contribution to our understanding of fertility behavior, the sine qua non of the Institute's program in population research.

Proposed Course: This study was funded for $77,424 in 1971, and extended with additional funds in 1972. It is expected to be completed in FY 1973.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Relationship between Age at Marriage, Birth Intervals and Total Fertility

Contractor: U.S. Bureau of the Census (reimbursable agreement)
Money Allocated: $165,000

Objectives: This project will permit the collection, tabulation, research and analysis of data on fertility of American women, with specific emphasis on data reflecting marriage, fertility and childspacing relationships. Designed to increase our knowledge of timing patterns of births and of factors that explain or bring about changes in timing, it includes research and analysis of current fertility levels and trends, the ways of using specific types of data for measuring changes in timing patterns of childbearing, ways of applying such knowledge to fertility projections, and exploration of new kinds of data needed to increase our understanding of recent fertility trends. A series of questions will be added to the Census Bureau's Current Population Survey questionnaire to obtain information on year of marriage, number of children ever born, birth order, and time interval between birth orders, to provide the basic data needed to develop childspacing patterns and to determine how they have been changing. The 50,000 household sample of the CPS will provide Nationwide statistics on current timing patterns as compared with corresponding data collected in former years (1960 and 1965).

Significance to Biomedical Research and Program of the Institute: This study will make an important contribution to the analysis of trends in fertility, by analyzing age at marriage and timing of births (childspacing) within marriage in relation to levels of fertility. An immediate objective is the development of a methodology applicable to fertility projection, but more broadly, it will also provide basic information for an understanding of fertility behavior and its changes in the United States over time. The project thus bears directly upon a primary concern of the Institute's program.

Proposed Course: This project is due for completion in FY 1973.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Mating Patterns in Seven Latin American Capital Cities

Contractor: University of Massachusetts
Money Allocated: $16,094 (1971)

Objectives: This study provides for the final tabulation, analysis, and interpretation of data from a fertility survey carried out in seven Latin American capital cities in 1964 under the guidance of the United Nations Demographic Center located in Santiago, Chile (CELADE). The survey produced data valuable for the investigation of: (a) female ages on first entering sexual union and (b) interruptions of sexual unions, both of these being immediate determinants of fertility. The research treats both age on entering unions and interruptions of unions as dependent variables. It seeks not only to determine their present levels and recent trends, but also to understand their determinants in order to better predict future trends.

Significance to Biomedical Research and Program of the Institute: This subject is clearly relevant to the Institute's program, since age at first entry into sexual union and interruptions in unions due to separations, widowhood, etc., are important factors affecting fertility.

Proposed Course: This project, initiated in FY 1970, will be completed in FY 1972.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Causes and Consequences of Sex-Age Differences in Human Populations

Contractor: University of California, Berkeley
Money Allocated: $42,488 (FY 1969)

Objectives: The purpose of this study is to collect data from as many countries as possible on the following: 1) sex ratio at birth, 2) sex ratios at successive ages, 3) sex differences in mortality, and 4) marriages by age of groom, to determine first of all, the statistical uniformities among these data and to explain them in social and cultural terms. A further objective is to experiment with models which will display the relationships between the various data when changes occur in one set of data (for example, the sex ratio) to see the extent to which they affect relationships among the other variables.

Significance to Biomedical Research and Program of the Institute: This study will have important policy implications and open new areas for further demographic analysis with respect to problems relating to natality differentials, disproportion of widows and widowers, varying marriage patterns and marriage markets, labor force composition, migration and other demographic matters.

Proposed Course: Work under this contract, begun in 1969, was extended without additional funds and will be completed in FY 1972.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Attitudinal Factors Affecting Family Size

Contractor: Worcester Foundation for Experimental Biology
Money Allocated: $46,657

Objectives: This research (1) tests the hypothesis that women who perceive little difference between sex roles and who reject (for themselves) the stereotype of women as less assertive, ambitious, intelligent, etc., than men are the ones most likely to accept employment and to desire and achieve relatively small family size; and (2) the "perceived sex-role differences and self-concepts" will be related to "antecedent biographical characteristics and family backgrounds." The second goal of the study will provide information for a later longitudinal study. The sample will include 1,000 persons--375 married men, their 375 wives, 125 single men and 125 single women of evenly distributed ages (17 to 55) and socioeconomic levels (based on husband's occupation or, for younger men, education). The subjects will be obtained from local civic, professional, religious, and labor union groups and will be selected to be representative of the community.

Members will fill out the questionnaires in group sessions. One questionnaire calls for rating 122 tested items three different ways, to diagnose male and female "sex-roles" as perceived for men, for women, and for themselves. The other questionnaire covers background data, employment and occupational history--including attitudes toward employment, achieved and desired family size, and contraceptive attitudes and practices. There are also questions about plans for working at the time of marriage, the factors that determined entry into the labor force or withdrawal from it, ideal family size and family planning orientation.

Significance to Biomedical Research and Program of the Institute: This study of several aspects of the relationship between achieved or planned fertility and the perception of the sex role of women has importance to the understanding of fertility behavior, which is crucial to the Institute's mission.

Proposed Course: The progress of this project has been very good. As a result, it was renewed to February 28, 1973. The level of first year funding was $40,089.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: An Investigation of the Factors Involved in the Moral Judgment of Marital Status and Family Size

Contractor: Human Resources Research Organization, Fort Knox, Kentucky
Money Allocated: $32,118 (1971)

Objectives: Some 500 middle class persons (100 of 16-17 year olds, 100 persons 18-20 years of age, and 300 adults divided among younger and older groups) drawn from ten organizations (PTA's, unions, alumni associations, Catholic groups, etc.) will be asked to respond to situation descriptions presenting marital status (single, married) and family sizes (0, 2, 6 children) under varied conditions of choice or choicelessness: personal desire, ability (power), and environmental circumstances. Respondents will describe their family status and how they achieved it. Various characteristics of the judges will be studied for their impact on judgments. Perceived attractiveness of the subjects judged will be examined (by bi-polar scale) to determine effects of moral judgment on this dimension. The study assumes that moral judgments are key factors in social pressures to conform, that single people and childless couples are widely viewed as blameworthy, and that this is deleterious to population control efforts. Analysis of variance will be performed to determine the effects of each of the three factors (from Heider) on moral judgment.

Significance to Biomedical Research and Program of the Institute: An understanding of population dynamics relative to future changes in the U.S. population requires a knowledge of attitudes toward family formation. This involves not only the couple but also the social context in which they live. Historically, parents, relatives, and friends have brought pressures on the couple to marry and to start childbearing early and to have large families. Thus it is highly relevant to the Institute's program to explore moral judgments as a framework for evaluating the effectiveness of programs designed to alter attitudes toward marriage or family size. The findings should be useful in counseling situations, family planning clinics, in wider educational programs dealing with marriage and family size, and in welfare programs. They should also contribute to improved maternal and infant health to the degree that they would foster changed attitudes and action programs designed to encourage the spacing of children at longer intervals.

Proposed Course: It is anticipated that this project will be completed within fiscal 1972, or early 1973.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Kin Network and Population Dynamics

Contractor: Harvard University
Money Allocated: $19,469

Objectives: This study will collect data on the extent and relevance of kin networks in the United States to determine their consequences on fertility and migration. It is hypothesized that the greater the intensity of the kin network (frequency of contact, etc.) and of the interchange therein, the higher the fertility. The kin network may also influence the amount and direction of migration. A conference of persons experienced in the family and fertility will be convened to approve and amend the trial questionnaire and another to evaluate the results. The pretest will be conducted by the U.S. Bureau of the Census.

Significance to Biomedical Research and Program of the Institute: Research on the relationship of the kin network to fertility has high program relevance. Very little work in this direction has been done.

Proposed Course: This project, initiated in 1971, is being conducted in conjunction with the Bureau of the Census which is administering a questionnaire to a sample of respondents in the monthly Current Population Survey. Completion is expected in FY 1973.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Effects of Purposive-Rationality, Traditionalism, and Economic Aspirations on the Process of Family Formation

Contractor: University of Kansas
Money Allocated: $44,000 (1971)

Objectives: This research specifies a unique data collection procedure involving hand delivering to the household questionnaires which are then self-administered and returned by mail. It would study 1,000 first-time parents to find factors affecting decision making as to the number and spacing of births. The independent variables are: (1) purposive-rationality, (2) traditionalism, and (3) economic aspirations as orientations affecting fertility (dependent variable). These independent variables should provide a parsimonious system with a high degree of explanatory power (e.g., as contrasted with the status variables of SES, education, and religion).

Significance to Biomedical Research and Program of the Institute: This study of how persons make decisions concerning family formation is definitely relevant to the Institute's program. The "training" of respondents to use a self-administered questionnaire facilitates followup over time and imparts to this study a methodological as well as substantive importance, since one of the problems with longitudinal studies is their very great expense. If this methodology works, it can serve as a model for other longitudinal studies at more reasonable cost.

The project should make contributions to the sociology of the family and be particularly helpful in understanding how and why persons decide to have different numbers of children. As a result, it should prove useful for persons in the area of family planning.

Proposed Course: This project, initiated in 1972 with three years' funding, is expected to be completed in FY 1973.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Determinants of Fertility, Migration, and Population Change of Counties in the United States, 1950 to 1960

Contractor: University of Georgia
Money Allocated: $81,781 (1971)

Objectives: This study will analyze the trends and patterns of population change for the total white and nonwhite populations of all counties in the United States from 1950 to 1960. Changes in this period will be explained in terms of fertility, mortality, and net migration, the major emphasis being on fertility and migration and upon the white and nonwhite differentials. The study also investigates the socio-economic and demographic determinants of each of the three components of population change among both white and nonwhites. County fertility rates and differentials, for example, will be examined by age (and age standardized), sex, color composition, and by levels of urbanization, industrialization, income, occupation, education, and related factors. Finally, tabulation and organization of the data by color, not previously compiled on a uniform basis will provide benchmark data for all counties in the U.S. for the 1950-1960 period for comparison with similar trends in the 1960-1970 decade. Plans have already been made for future computation of the 1960-1970 components of county population change by color. Maps will be prepared showing county birth, death, and net migration rates, and natural increase rates, by color. Birth rates of counties which gained and lost population will be systematically examined, and constellations of counties with similar fertility and net migration patterns will be identified.

Significance to Biomedical Research and Program of the Institute: This efficient utilization of massive data already processed, and coupled with further data from the censuses and the county and city data books, for the 1950's is a useful approach to the analysis of population change in clusters of counties and regions of the United States, and is basic to a projected analysis of the dynamics of the 1960's. This study is exceedingly relevant to the study of population processes in the United States and, therefore, to the Institute's program, both from a descriptive and an analytical point of view.

Proposed Course: This project is in its second year and is expected to be completed during fiscal 1972.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: An Analysis of the Spacing of Pregnancies, Births, and Completed Fertility in Relation to Socio-economic Status, Intergenerational Mobility and Aspirations for Children Aberdeen, Scotland

Contractor: Brown University
Money Allocated: $48,469 (1971)

Objectives: The data are based on a probability sample of resident women of Aberdeen, Scotland, who had at least one live birth during the period October 1950-September 1955. The pregnancy histories of these women up to 1964 have been copied from hospital records and will be updated to 1970; the other required information has been obtained by means of an interview survey (2,000 cases). Preliminary tabulations of the survey data indicate that a "significant test of intergenerational mobility hypothesis" is possible with the data at hand and that the "data on mobility aspirations for children are particularly exciting and promising."

The study will investigate: (1) how spacing of pregnancy varies with the type of terminations of previous pregnancies, the stage in the family life cycle, age at marriage, age at first pregnancy, and socio-economic status; (2) how the spacing of live births, the number of children ever born, and the length of fertility span vary by age, age at marriage, age at first pregnancy, socio-economic status, social mobility, and mobility aspirations for children; (3) the life conditions under which women are likely to resort to terminations and sterilizations; and (4) how the size of the family of origin, and position on a traditionalism scale are related to social mobility, present socio-economic status, aspiration for children and fertility.

Significance to Biomedical Research and Program of the Institute: The Aberdeen data provide a rich source to know how the outcome of one pregnancy influences subsequent pregnancies, how family origin variables relate to childspacing, and how fertility relates to social mobility. The analysis of pregnancy histories and the relationship between childbearing and social mobility are thus very relevant to the Institute's program of population research.

Proposed Course: This study, initiated in 1970, is expected to be completed in FY 1973.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Socioeconomic Status and Family Factors in Residential Mobility

Contractor: U.S. Bureau of the Census (reimbursable agreement)
Money Allocated: $4,982.98 (1971)

Objective: This study will combine data from the March Current Population Survey taken by the Bureau of the Census for the years 1966-1970 to study annual migration during that period by single years of age. It is designed to provide new perspectives on the joint influence of various socioeconomic and familial statuses on the probability of making different types of moves. It will also produce statistics allowing more precise estimates to be made of the number of times an individual would move in his lifetime if he moved according to currently existing age-specific rates of residential mobility. The study will also examine the effects of number and ages of children (simultaneously considered) on the probability of moving. In these comparisons, age of family head will always be controlled, and so will occupation in some cases. Other questions to be investigated include the effect of the wife's labor force status (whether or not she has a job) on the family's geographic mobility when age and occupation of husband are held constant. A related question is the overall effect of occupation on geographic mobility for men and women, when age and marital status are held constant. In most cases the effects of the various socioeconomic and family statuses will be examined for the Negro and white populations separately. Comparison of the annual data on moves with data from the 1970 census based on residence in 1965 and 1970 will be possible.

Significance to Biomedical Research and Program of the Institute: This research will produce information on characteristics of migrants not previously assembled, making possible more refined estimates of probabilities of moving each year, by occupation, education, race, and family characteristics. It is a carefully planned project by a competent investigator, at marginal cost. Study of migration differentials is relevant to the Institute's program, especially as these relate to family status and size of family.

Proposed Course: It is expected that the work on this contract will be completed within fiscal 1972 and that there will be no need for extension or renewal of the contract.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Migration, Employment and Earnings in the States, Metropolitan Areas, Non-Metropolitan Areas, 1957-62 and 1962-67

Contractor: U. S. Department of Commerce (reimbursable agreement)
Money Allocated: $22,600

Objectives: Data from the one-percent sample of reports on the Social Security-covered workforce will be tabulated to provide a data bank from which other investigators can study mobility among the States, SMSA's, and nonmetropolitan areas of the United States. These tabulations would measure inmigration, outmigration, and the nonmigrant workforce of each relevant area, cross-classified according to age, race, sex, area of origin (for inmigrants), area of destination (for outmigrants), industry of employment and level of earnings. The tabulations will show the employment and mean wages for those working in each area according to status as inmigrants, outmigrants, new entrants into the workforce, and retirees. From such tapes changes in employment in any one of these areas may be analyzed with reference to previous employment the year before or for a period of years (1) in that area or (2) in any or all of the other areas, and distinguished by the following variables: (a) all employment (b) industry classification, (c) age, (d) sex, (e) color, and (f) average wage in the current and previous employment.

Significance to Biomedical Research and Program of the Institute: The redistribution of population and related factors associated with population movements are of the highest relevance to the Institute's program. No other data are as good, and the publication of a good set of tabulations from the OASDI one-percent sample will be a milestone in migration research. The tabulations will be particularly useful in determining the relationship between migration flows by age, color, and sex with respect to economic opportunities both between the states and between particular SMSA's, and nonmetropolitan areas. For example, it will be possible to analyze for such a city as Chicago the sources of migration from other parts of the United States by age, sex, color, and some of the economic factors which underlie these movements.

Proposed Course: This reimbursable agreement was initiated in 1971 and it is expected that work will be completed during fiscal 1972.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Distribution and Differentiation of Population Within Metropolitan Areas

Contractor: University of Michigan
Money Allocated: $110,936 (1971)

Objectives: This study will analyze 1970 U.S. census data on tapes for 20-25 SMSAs and compare these with census materials for 4 Canadian metropolitan areas to determine: (1) the degree of residential segregation of different socio-economic groups; (2) the degree of segregation of families in different stages of the life cycle; (3) the pattern of segregation of housing of different types and ages; (4) the relation of housing type to socio-economic characteristics of occupants; and (5) variations in population distribution in different metropolitan areas.

The distribution and differentiation of population in cities is of major importance, but to date there have been few empirically based studies on this topic. This study will provide a framework for considering the similarities and dissimilarities of 25 cities, which is a sufficient number of the Standard Metropolitan Statistical Areas to give meaningful results. In the process it will develop generalized analytical tools for the study of other groups of cities. This research is more than just a study of segregation patterns and looks at a number of associated factors including socio-economic groups and structures and life cycles in relation to housing.

Significance to Biomedical Research and Program of the Institute: This study will have a great deal of explanatory power concerning the population dynamics of our urban communities, and the Canadian data will offer an interesting comparison. Urban planners should find the project's results useful. Thus, this research is extremely relevant to the Institute's goals.

Proposed Course: This project, begun in FY 1971, is expected to be completed in FY 1973.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Rates and Consequences of Population Change: The Growth and Decline of Counties in the Mountain Region

Contractor: Colorado State University (Fort Collins)
Money Allocated: $85,480 (1971)

Objectives: This study will describe the population changes that have occurred in the Mountain Region (8 States, 279 counties stretching from Montana to Arizona) during the period 1950-1970. Almost one-half of these counties lost population between 1950 and 1960 and more than one-half declined in population during the decade 1960-1970. The main interest centers on the consequences of population change--consequences to public finances, retail activity, housing utilization, and agricultural activity.

Censuses of population, governments, businesses, housing and agriculture, and other published and unpublished pieces of information (available from State and local sources) will be used. Components of change as well as some indices of social welfare, such as doctors per capita, use of health services or school retardation, and grade continuation rates will also be explored to clarify some of the relationships and problems being investigated.

Significance to Biomedical Research and Program of the Institute: This study is both worthwhile and program relevant. Depopulation is one of the more important, yet neglected, aspects of the population problem in the United States. Most of this Nation's counties either lost population or experienced net outmigration in the last two decades, and very large geographic areas are coming to have sparse populations. Few studies have investigated the process or the consequences of such population losses.

Proposed Course: This study, funded in 1971, is expected to be completed in FY 1972 or early FY 1973.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: An Economic Analysis of Migration from Rural Eastern Kentucky to Selected Urban Centers
Contractor: University of Kentucky
Money Allocated: $61,000 (1971)

Objectives: The purpose is to measure the impact of migration in economic terms on individual donor and recipient local governments and the Federal Government. It will seek to measure and analyze the cost and returns of population relocations through migration from eastern Kentucky to recipient metropolitan areas in Kentucky and Ohio and to isolate the major socio-economic variables giving impetus to this movement. It will include analyses of both initial migration and return migration and the marginal cost of providing selected public services in high density neighborhoods in the cities studied.

Significance to Biomedical Research and Program of the Institute: The evaluation of the consequences of migration is especially relevant to the program of the Center for Population Research, which has as one of its emphases the study of the consequences of population growth and change. It will provide information of concern to the Public Health Service in that it will document the cost to both donor and recipient communities of migration within the United States.

Proposed Course: This contract, initiated in FY 1970, will be completed in FY 1972 or early FY 1973.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Demographic Transition Without Urbanization: Rural Fertility Reduction in the Southern Philippines
Contractor: University of Colorado
Money Allocated: $21,139 (FY 1970)

Objectives: The purpose is to determine the extent to which fertility of migrants to essentially rural areas may be affected by the type of economic and social arrangement in the new area. The investigator will study four communities of migrants in the Philippines, two of which can be categorized as innovative (i.e., encouraging the development of new forms of social interaction motivated by the desire for profit) and two that may be categorized as replicative (i.e., similar in social structure and way of life to the area from which the migrants came) to determine to what extent fertility in the former localities or communities is lower than in the latter type communities.

Significance to Biomedical Research and Program of the Institute: A finding that fertility can be lowered without urbanization, and the specification of some of the conditions under which this happens, would be of immense significance to the formulation of population policies throughout the world.

Proposed Course: This study, begun in fiscal 1969, is expected to be completed in fiscal 1972.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Population Policy-Related Attitudes and Values
Contractor: Carolina Population Center
Money Allocated: $27,062

Objectives: This study will assess by several methods the attitudes toward specific and general population policies in the area of fertility and population growth, and the salient values which are related to, or involved in, the proposed policies, with a view toward policy development and application. More specifically, the investigators will: (1) determine the knowledge and attitude of three groups--(a) legislators, (b) key leadership groups, and (c) constituent groups, toward population policies; (2) measure the underlying value structure of the attitudes; (3) explore the similarities among the attitudes and values of the three groups studied; and (4) generate suggested programs for developing and expediting population policies.

The study will develop accurate and valid methods of (a) measuring knowledge, attitudes, and values of the three groups; (b) identifying members of these groups; and (c) comparing the three groups on relevant measures.

Significance to Biomedical Research and Program of the Institute: This research will make a technical and methodological contribution to the study of attitudes held by sub-groups toward population values and policies. As such, it is needed research in a very significant area that is highly relevant to the Institute's interests regarding the development of population policies.

Proposed Course: It is anticipated that this project for a pilot study, initiated in 1971, will be completed within fiscal 1972.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Effect of Income Payments to Reduce or Increase Natality

Contractor: University of Illinois
Money Allocated: $95,076

Objectives: This study will explore the effect of income on fertility in two different studies: (1) by asking supposititious questions in a sample survey administered to a regional sample of the U.S. population concerning the effect on personal fertility of government payments for more or fewer children; and (2) by studying the relationship between income and fertility using the 1 in 1,000 tape from the 1960 census, using multiple regression techniques within fine sub-classifications and interpreting results with the aid of demand theory.

Significance to Biomedical Research and Program of the Institute: The relaship between income and fertility comprises a critical area of research and thus is highly relevant to the Institute's program.

Proposed Course: The contract, initiated in fiscal 1971, is expected to be completed in fiscal 1972.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Psychological Studies of Social Norms Influencing Family Size

Contractor: University of Kansas
Money Allocated: $102,825

Objectives: This study will investigate the dynamics of social norms concerning large and small families--reactions to explicit and implicit social pressures in relation to normatively defined family size ideals. The following laboratory experiment is proposed: positive and negative evaluations of women as a function of the number of children (0-5) they have in relation to their age (25, 35, and 45) and career types (none, child-related career, non-child-related career, and non-child-related career with high career commitment). In the laboratory situation a sample of white, middle class, married women will evaluate women based on these variables in a design in which they will not be aware that these are the crucial variables in the experiment.

Significance to Biomedical Research Program of the Institute: The study of the dynamics of social norms concerning large and small families is highly program relevant, the experiment approved b the panel are feasible, and the methodology of getting at the variables is straightforward. The investigator has had considerable experience with experimental social-psychological research, and the budget is reasonable. For these reasons this study merits approval.

Proposed Course: This study is scheduled to run for only one year rather than originally proposed two years.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Support of Publication of Population Index

Contractor: Princeton University
Money Allocated: $86,378

Objectives: The purposes of this support are to permit the continuation of the publication of Population Index as a quarterly journal containing bibliographic information and special articles in the population field; to computerize the preparation of final copy for photo-off-set reproduction of the Index; and to develop a computerized retrieval service for the production of special bibliographies.

Significance to Biomedical Research and Program of the Institute: The need for knowledge about changes in population size and composition and the way in which these are related to socio-economic factors is well recognized by policy makers in the field of public health. For these and other users of demographic resources, an accurate and comprehensive guide is essential. NICHD funding permits the maintenance of a bibliographic tool of tested utility and the broadening of its usefulness for searching and finding. The Index over the past thirty-five years has also carried a broad range of articles on current developments in demography.

Proposed Course: This contract, initially funded in 1971, is being continued.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Occupational Experience as a Determinant of Fertility Among American Women

Contractor: Center for Policy Research
Money Allocated: $205,980

Objectives: The negative relationship between women's participation in the labor force and their fertility is well established. Previous studies, however, have analyzed the relationship between women's working and fertility in terms of participation and non-participation. The proposed study will take a more sophisticated strategy by considering the characteristics of the work women do and the relationship between these occupational characteristics and fertility. The study's main goal is to determine whether differences in occupational opportunities and rewards result in variations in the fertility behavior of women.

The study will use published data from the 1940, 1950, 1960, and 1970 censuses. The bulk of the analysis will not be based on published material, but rather will be based on the one percent Public Use Sample tapes of the 1960 and 1970 censuses. The 1970 one per cent tapes will be especially useful since the interview schedule contained a number of retrospective questions concerning the occupation of each household member in 1965. Moreover, there are a number of questions on the subject's marital history and number and age of children. Lastly, the tapes will have an occupational code which has a high level of specificity that will allow the researcher to categorize occupations in the most suitable theoretical and methodological manner.

Significance to Biomedical Research and Program of the Institute: This project will make valuable contribution to the population field. The principal investigator intends to examine an important aspect of the relationship between women's participation in the labor force and their fertility behavior, namely, the chracteristics of the occupations they work in. Recently the changing role of women has become more pronounced. Indeed, some persons have termed this change as a "social revolution." There are indications that a large number of women are taking or attempting to take a more active role in the labor market, especially with professional occupations. Currently, it is not known how the differential participation in the labor market or different sectors of the labor market influences fertility and social institutions, such as the family. This project would do much to better understand contemporary and future changes.

Proposed Course: This contract was initiated in FY 71 and will run for a two year period.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Correlates of Family Size and Child Spacing in the United States and Puerto Rico

Contractor: Boston College
Money Allocated: $150,612

Objectives: The purpose of this two-year study of 500 families of high school students in a middle-class suburb of Boston is to show the effects of family size and the spacing of children on the "quality of life" of the parents and of the children. Variables on the parental side include: age, socio-economic status, religiosity, education, work history, childbearing attitudes, recreational patterns, financial conditions, membership in voluntary organizations, degree of integration into the community, and rationale for birth of each child. Variables on the children's side include: academic achievement, intellectual capacity, vocational and educational aspirations, personality, social relations, work history and expected age at marriage, family planning attitudes and subsequent life style. Interviews and questionnaires in addition to peer ratings and school records of the children are being used as sources of the information. This project replicates the key aspects of another NICHD project conducted in a middle-class suburb of San Juan, Puerto Rico, by the same co-investigators. New variables are being added in this study, including child spacing and the impact of family size on the lives of parents.

Significance to Biomedical Research and Program of the Institute: The analysis of family size and birth spacing is crucial to the program of the Center for Population Research, NICHD, and particularly as this study looks at these aspects in a cross-cultural context.

Proposed Course: This contract is funded for two years, November 1971 to November 1973.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Family Career and Sexuality

Contractor: Center for Policy Research
Money Allocated: $43,421

Objectives: The purpose of this research is to examine the possible relationship between ideology and fertility related behavior. Specifically, the study includes the development of a quantitative model which will examine the relationships between: 1. family background, 2. family ideology (the attitudes and values of the respondent's mother and father), 3. the respondent's ideology, 4. the respondent's career and family role orientations, 5. sexual attitudes, 6. sexual behavior, and 7. preferred family size. The model will be developed through the analysis of data already available. There are 4 data sources: First, an Ideology Study of 668 students attending the University of California, Berkeley, in 1970. Second, a similar survey of Berkeley students' parents, entitled Intergenerational Study. Third, a National Student Study sponsored by the Carnegie Commission and consists of approximately 71,000 college students. And, fourth, a National Adult Study which has data similar to that collected in the Intergenerational Study.

Significance to Biomedical Research and Program of the Institute: This project would deal with a new way of analyzing fertility behavior. The model resulting from this project should encourage other researchers to examine population dynamics from a new perspective.

Proposed Course: It will require one year to conduct the secondary analysis outlined in this study.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A Study of Optimum Population Levels

Contractor: University of Virginia
Money Allocated: $63,070

Objectives: This study is exploring different approaches to, and a methodology allowing the calculation of, an "optimum level of population" in terms of increases or decreases in an index of quality of life (IQL). The major effort is toward a modification of the Gross National Product (GNP) to adjust for the amount of the national income accounts which are directed to the diseconomies produced by population growth. The study will attempt to project changes in the so-called IQL as population increases and as population distribution changes under the assumption of various technologies relating to mineral consumption, water use, energy, and other natural resources.

Significance to Biomedical Research and Program of the Institute: This is one of the very few studies in the Behavioral Sciences program of the Center which addresses itself to the problem of measuring the consequences of population growth and change. Much of the current concern over the effects of increasing population size has been based on data and on assumptions which have not always met with general acceptance. This study is attempting to develop a methodology which will make the consequences of population growth and change much more specific and identifiable, at least in economic terms, and therefore should be of great benefit to policy makers in the determination of what population goals should be pursued.

Proposed Course: This study was funded for one year in fiscal 1972 with the anticipation that it would be renewed for at least two additional years.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Demography of the Black Population

Contractor: University of Texas
Money Allocated: $90,528

Objectives: The purpose of this study is to describe and analyze trends in the demographic characteristics of the black population, and compare these with trends in the white population. The basic analytical approach will be a cohort analysis by sex and color. The principal investigator did a related study for the 1960 census, Changing Characteristics of the Negro Population: A 1960 Census Monograph, U.S.G.P.O., 1969. Part of this project will be to check the projections outlined in this earlier work, seek to explain changes from 1960 and 1970, and to update the earlier monograph. The principal investigator will focus on how changes in social policies, e.g., the Civil Rights Act of 1964, may influence changes. Projections will be made for future trends. Although the period from 1960 to 1970 will be emphasized, the 1930, 1940, and 1950 censuses will also be utilized. Topics to be dealt with are: income, education, home ownership, place of residence by region, urban and rural, relationship to head of household, employment status, and the beginning of a series on region of residence by state of birth, migration, population distribution, occupational structure and change, and marital patterns and household composition.

Significance to Biomedical Research and Program of the Institute: The dynamics of the black population are not that well understood and, until relatively recently have not been systematically analyzed for their own value. Rather, the demographic changes of the black population were considered in a secondary manner, as something to compare the white population with. This project, of course, will take the opposite tack and analyze the black population, considering the white population only as a reference point for differential change. This project will consolidate what we know about the dynamics of the black population. There is sizeable literature on blacks and a fair number of reports on the demographic aspects of the blacks. The reports, however, usually concentrate on a particular subject and/or region, e.g., black migration to the North and their levels of income. This report would take a holistic approach to the black population.

Proposed Course: This is the first of two year's funding.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Population Redistribution and Depopulation in Non-Metropolitan Pennsylvania: Social and Economic Correlates and Policy Implications

Contractor: The Pennsylvania State University
Money Allocated: $313,833

Objectives: This is a complex undertaking which will investigate in detail, historically and contemporaneously, the non-metropolitan population changes in the State of Pennsylvania. That State is a large mature area with a highly diversified non-metropolitan sector. The investigators propose to create a data bank of relevant historical-statistical information and to create a typology for the non-metropolitan areas in order to analyze the most important processes shaping change in those areas. Data will be derived primarily from the census but several other sources will be used and field studies will be conducted. The studies will focus on transport, technology, changing labor force roles of females, attitudes toward metropolitan living, and others - which have shaped the non-metropolitan sectors of the population.

Significance to Biomedical Research and Program of the Institute: This proposal will differ from earlier research on related topics in demography and related fields by its strong focus on a great range of non-metropolitan characteristics and trends in a highly advanced region. The issue of population dynamics in non-metropolitan areas affecting population distribution has been a very neglected area for research. Several proposals are currently pending in Congress and elsewhere fro increasing the attractiveness of non-metropolitan areas. Whether or not these are well advised depends upon the understanding of the forces and processes affecting such areas and upon projected distributions of population and economic opportunities.

Proposed Course: This multi-faceted research program will extend over a period of two years.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Analysis of Migratory Responses to Employment Opportunities

Contractor: Battelle Columbia Laboratories
Money Allocated: $54,885

Objectives: The aim of this research is to clarify the relationship between migration and certain demographic and economic variables and in particular potential employment, i.e., differential migration in response to economic conditions. For a sample of 100 counties, drawn from a 14-state area, in-migration rates for 1955-1960 can be obtained from a special tabulation of the 1960 census. Rates of net-migration by area, sex, and color, will be obtained from the Bowles-Tarver series and, after adjusting for a five-year period (instead of the decade over which they were computed), rates of out-migration will be obtained by subtracting rates of in-migration from adjusted rates of net-migration. With age, sex and education controlled, a clearer relationship between migration and economic opportunity should emerge. Migration rates for each group will be regressed on variables measuring aggregate employment opportunities and group specific employment opportunities in county labor market areas. The 1955-1960 data will be used to predict 1965-1970 migration and will be checked against cross-tabulated data from the 1/100 sample for 1960 and 1970.

Significance to Biomedical R search and Program of the Institute: The proposed research has high program relevance, fitting into a mosaic of our existing contract migration studies relating to: (1) the development of the Social Security file covering 90% of employment in the United States to obtain data on inter-regional, metropolitan, and non-metropolitan migration patterns by sex, color, and age: (2) migration from Appalachia, and (3) the impact of depopulation upon economic and social institutions in the Rocky Mountain area. The present research proposal relating migration and economic opportunities should make a real contribution to the understanding of the determinants of migration, a subject of increasing policy interest.

Proposed Course: This project has been approved and scheduled for a one year period.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Review of Research Findings on Rural-Urban Migration with Annotated Bibliography

Contractor: TRACOR, Inc.
Money Allocated: $36,175

Objectives: This project was originally funded by OEO. OEO has decided not to support projects in migration and, as a result, discontinued its support. OEO's discontinuation does not reflect negatively on the project.

Work will continue on the development of an annotated bibliography and review of all research on rural-urban migration. The principal investigator proposes to annotate items in the existing bibliography, add more items, expand the summary of research findings, and suggest needed research.

Significance to Biomedical Research and Program of the Institute: This project is highly relevant. Such publications save established investigators a lot of time and money. Perhaps more importantly, such a work can make it easier for students to enter this field of research. Thus, such a project has an extended utility not found in many research projects.

Proposed Course: This project is scheduled to run for a one year period.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A Study of the Growth of Mexican-American Families

Contractor: University of California at Los Angeles
Money Allocated: $71,811

Objectives: The objective of this research is to design andcarry out an initial and exploratory analysis of the determinants of fertility and family planning among Mexican-Americans in Los Angeles county. Next to Blacks, Mexican-Americans constitute the most important minority in the United States and have the highest fertility of any major cultural or ethnic minority. The study emphasizes the effects of social structure and social norms on fertility and family planning but also considers social-psychological variables. A multistage probability sample of 1200 Spanish-surname women of childbearing ages is being interviewed to collect the following data: (1) reproductive experience, including a history of births and pregnancies as well as number of children expected or desired; (2) past and anticipated contraceptive usage; and (3) determinants of both reproductive and contraceptive experience. More specifically, these determinants include knowledge of, and attitudes about, contraceptive practices and family size, extent of orientation toward or involvement in Mexican-American society, socio-economic status and social mobility, family structure, characteristics of community residence, migration history and religious practices. Various multi-variate techniques will be used to analyze the data.

Significance to Biomedical Research and Program of the Institute: The Mexican-American minority is of particular interest because it comprises one of the minority groups and enclaves of high fertility in a nation with generally declining fertility.

Proposed Course: This contract is funded from June 30, 1971 to September 29, 1972.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Explorations in Fertility Values

Contractor: Cornell Aeronautical Laboratory, Inc.
Money Allocated: $65,289

Objectives: The main purpose of this project is to develop and demonstrate methods to reveal how fertility values are related to family size preferences. The principal investigator is currently developing ways in which family size preferences and the desired number of children under various economic, social, and psychological conditions can be quantified. Once the methodologies for these two tasks are refined, it will enable other investigators to measure accurately the number of children persons wish to have under various situations. Moreover, it should give insight into how people come to desire different numbers of children at different points in time.

The study entails two surveys of 300 white, married women in the 15-44 age range. For each major religious group (Protestant, Roman Catholic, and Jewish) there will be 100 women represented.

Significance to Biomedical Research and Program of the Institute: This project examines the socio-psychological factors which influence individuals' desired family size under different situations and in different points in time. This type of study has great potential in assisting family counselors in helping individuals plan their family and in assisting individuals who may be experiencing familial problems as a result of too many or too few children.

Proposed Course: This is a two-year contract. The first year ends June 30, 1972. The first survey has been completed and the data are currently being analyzed. The second survey will be done during the contract's second year. The level of funding for the second year is $127,624. The end product of this project will be a model which shows the relationship between fertility values and family size preferences. The measuring instruments developed under this contract should prove quite valuable for other researchers who undertake larger regional or national surveys in this area.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Role in Fertility Patterns of Urban Mothers

Contractor: Columbia University
Money Allocated: $88,828

Objectives: This project is exploring the relationship between female roles and fertility. The major hypothesis of the study is that timing of the first birth greatly influences the number and timing of subsequent births, the manner in which a woman views herself in relationship to familial roles (i.e., mother and wife), and how she perceives herself in non-familial roles, (e.g., student and worker). The principal investigator is conducting an exploratory study of women in the New York area between the ages of 15 and 29 who have recently bore their first child. The research instrument concentrates on the determinants and timing of the first birth. Specific areas to be explored are the socialization process the subjects underwent in terms of their family of origin, friends, social economic status, religion, race, and their perception of their mother and father. This project is innovative in the sense that most studies which deal with fertility and, especially those that try to project future fertility patterns, have concentrated on the most recent birth which may or may not be the last birth. It is also a methodological study which will develop a research strategy and instrument which, if successful, will be utilized in a larger study.

Significance to Biomedical Research and Program of the Institute:

This project has great potential for increasing our knowledge of the factors which determine fertility at the individual's level. As a result, the information which may ultimately come from the project will be valuable for the medical and allied professions in advising persons how to better plan their families.

Proposed Course: This is a one-year project which was initiated on March 15, 1972. At the end of the contract period, the research instruments and findings of the pilot study will be evaluated in order to determine if further research in this area will be fruitful.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: A Final Household Survey to Complete the Family Planning Evaluation Project

Contractor: University of North Carolina (Chapel Hill)
Money Allocated: $200,088

Objectives: The primary purpose of this project is to allow completion of a large-scale controlled action experiment of an area sample survey of 16 American cities involving a total sample size of about 3200 ever-married women in order to evaluate the effect of family planning programs on the fertility and fertility behavior of communities. This post-survey was made in each city in 1969. Data from the two area sample surveys of ever-married women living in low-income census tracts will be integrated with other data from monthly interviews of newly-delivered mothers, quarterly vital statistics tabulations, operating statistics of the projects, and other data resources (all paid for by other grants) for final interpretation of the impact of family planning programs and the reproductive behavior of the communities they serve. Effects are being measured on four levels: knowledge and attitudes toward family planning, use of services, contraceptive behavior, and reproductive events.

Significance to Biomedical Research and Program of the Institute: Although this project has operational research aspects not described here, it is basically examines the premises on which family planning projects operate, and their consequences for the behavior of communities. The initial survey was funded by a grant from Maternal and Child Health Services (MCHS) of the Department of Health Education and Welfare, but its grant funds cannot appropriately be spent for any part of the work on a survey dealing with population or its analysis. Moreover, the National Center for Family Planning Services is concerned with strictly operational research in specific clinic situations rather than with the evaluation of family planning in the broad sense. Thus, the Center for Population Research, NICHD is the most appropriate agency to fund this study.

Proposed Course: This contract is funded for two years, from June 1972 to June 1974.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Social and Psychological Factors Affecting Fertility, Family Planning and Clinic Utilization

Contractor: Wake Forest University
Money Allocated: $95,936

Objectives: The purpose is to re-analyze in-depth data collected in three KAP-type interview surveys concerned with black males and females in Winston-Salem, North Carolina, and Hackensack, New Jersey, and with Spanish females in Costa Rica. Emphasis will be placed on psychological and social-psychological variables as they relate to fertility, family planning and clinic utilization. For women with a high risk of high fertility various social-psychological measures will be devised and related to different sets of explanatory variables. Twelve different analyses will be undertaken to test out hypotheses.

Six analyses will pertain to black women in the United States regarding: (1) definition and indexing of the context of "risk" (biological, societal, and medical); (2) early sexual experience and attitude toward sex; (3) comparison of male-and-female-headed black households as to differences with respect to such factors as education, parity, number of current sex partners, and attitude toward abortion; (4) poverty as a "culture of poverty" vs. the relationship to intervening variables, such as education, age at first marriage, legitimacy of first conception, and age first learned to prevent conception; (5) religion, social class and abortion; and (6) consequences of being born out of wedlock.

The six analyses pertaining to Costa Rican women will be concerned with such aspects as: (1) modernization and modernism; (2) correlates of fertility; (3) trends in child spacing; (4) credibility of sources of family planning; (5) urban-rural differences in felta and infant mortality; and (6) comparison of consensual and legal marriages.

Significance to Biomedical Research and Program of the Institute: This project is relevant to the NICHD program relating to the interface between population and family planning in that it goes beyond the clinical approaches taken by such governmental agencies as AID, and the National Center for Family Planning Services. It also brings in a cross-cultural aspect, which relatively few studies have done.

Proposed Course: This contract is funded for two years, beginning June, 1972.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Determinants of Fertility Change in Tamil Nadu, India

Contractor: University of North Carolina
Money Allocated: $192,298

Objectives: This study aims to investigate whether there is a threshold of social and economic development after which family planning action programs will make more rapid progress. The approach proposed is "observational" rather than experimental. The existing geographic differences in social and economic development and in family planning action program inputs in one state (Tamil Nadu) in India will be used to select an appropriate sample of areal units (Community Development Blocks and Hamlets), and the fertility and family planning behavior of the populations in these units would be studied.

The total project has two parts. In Part 1, the available data for all 375 Community Development Blocks in Tamil Nadu would be compiled and analyzed to assess the variation in fertility or contraceptive acceptance attributable to: (a) differences in family planning program inputs, (b) differences in socio-economic level of development, and (c) interaction between program input and level of development. These data would also be used in selecting a sample of Blocks for Part 2. Within each of the 54 sample Blocks 12 Hamlets (of 1,000 population each) would be selected. All the sample units would be drawn so as to represent all combinations of program inputs and levels of socio-economic development ot permit repeating the analysis in Part 2 with more accurate data on program outputs.

Significance to Biomedical Research and Program of the Institute: This study affords an excellent opportunity to investigate whether there is a threshold of social and economic development above which family planning action programs may make more rapid progress.

Proposed Course: This proposed research has been scheduled to run three years.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: An Analysis of the Causes and Patterns of Rural to Urban Migration of the Poor

Contractor: University of California (Berkeley)
Money Allocated: $16,632

Objectives: This study will investigate the causal structure and decision making process underlying the migration behavior of the rural poor and explore the potential for various public policies which could influence migration from rural and small areas to big city ghettos. The study will test a series of alternative causal models for explaining migration while investigating in depth a model of individual adjustment to economic and social displacement. The principal investigator will conduct a secondary analysis of data previously collected by ABT Associates and funded by OEO. To date, the analysis of these data have been only cursory. Data for 1800 lower socio-economic respondents have been collected. Approximately half of the study population were migrants from rural to urban areas, and the remaining were current residents of rural areas. Three migrant streams were involved: Southeastern Blacks, Appalachian Whites and Southwestern Spanish Americans. The data collected include information on perceptions, experiences, and attitudes as well as migration behavior.

Significance to Biomedical Research and Program of the Institute: The study population is important in terms of explaining the causes of migration patterns of the poor. Economic and other pressures which may force poor families to migrate to urban areas has implications for the planning and implementation of public health programs.

Proposed Course: The contract, initiated in fiscal year 1972 is anticipated to run for one year.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Employed Woman: Family Planning and Careers

Contractor: University of California at Los Angeles
Money Allocated: $36,458

Objectives: The purpose of this proposal is to elucidate the behaviors and motivations of 120 employed career women relating to fertility and family planning and to specify the patterns of temporal ordering of family and work. Psychological motivations for having children will be delineated and the intensity for desire for children, versus intensity of desire for various alternatives to childbearing will be compared for each group of women. One important facet of the research will consist of an attempt to quantify the perceived rewards and cost of both the motherhood role and the career role. Data analysis will be structured to relate directly reward cost data to fertility variables such as actual number of children and expected number of children to career variables such as length of full-time employment, personality and developmental factors, that may influence the differences in employment history, fertility and desire for children.

Significance to Biomedical Research and Program of the Institute: This study represents an excellent opportunity to get family limitation information from a group of employed career women.

Proposed Course: The contract initiated in 1972 will run for one year. At the end of year one, the contract may be continued to allow the investigator to conduct a longitudinal study of a subsample of 40 subjects.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Fertility of the Spanish-American Populations in the United States and Their Socio-Economic Correlates

Contractor: Research Institute for the Study of Man
Money Allocated: $258,110

Objectives: Using data from standard and special 1970 and earlier dicennial census tabulations, this study will examine the fertility and its socio-economic correlates of the major Spanish-American population groups in the U.S.: Mexican, Puerto Rican, Cuban, Central and South American and the descendants of the original (17th and 18th century) Spanish group. The 10 million Spanish-Americans living in the United States comprise about 5 percent of the total U.S. population--or the equivalent population of the sixth largest Latin American country.

Significance to Biomedical Research and Program of the Institute: This is an important research effort which will further explain fertility differentials between major Spanish-American population groups. The effort to look at different sub-groups of the Spanish-American group (comprising 5 percent of the total U.S. population) will be useful in identifying cultural differences or differences due to processes of integration into the American culture.

Proposed Course: This complex secondary analysis of data is scheduled to take three years to complete.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: College Women in the 1950's: Attitudes and Behavior with Respect to Marriage, Children, Divorce, and Sexual Practice

Contractor: The Wright Institute
Money Allocated: $16,067

Objectives: The purpose of the proposal is to analyze data that are available on 3,180 Vassar College women during the period 1953 through 1960. These women participated in the Minnesota Multiphasic Personality Inventory, the California Psychological Inventory, the Vassar Attitude Inventory, and responded to several other standard questionnaires relating to such topics as marriage, children, career plans, sexual behavior, etc. Based on these data the following hypotheses will be examined. (1) in the years 1953-60 almost every Vassar student aspired to marriage; (2) almost every student aspires to motherhood; (3) almost no student expresses interest in adoption unless a physical condition hampered pregnancy; (4) almost no student expresses concern with problems of overpopulation. The above represents four of 15 marriage and fertility related issues which will be examined in this secondary analysis.

Significance to Biomedical Research and Program of the Institute: This study may reveal many of the current revoluntionary themes which may have been latent in the 1950's. For example, women's lib, sexual freedom, decline of the number of children desired, etc., may have been in their embryonic form.

Proposed Course: This investigation of secondary data is scheduled to require a period of one year.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Migration of the Aged and Kinship Structure

Contractor: University of Missouri
Money Allocated: $51,434

Objectives: This project is concerned with the kin network system from the perspective of the elderly (persons 65 years of age or older), with special emphasis on the ways in which migration influences the system. Specifically, this research will: 1. determine the subject's residential proximity to kin; 2. the extent and nature of their interaction with kin; and 3. how these relationships were arrived at. To obtain their goals, 800 persons will be interviewed in Kansas. The subjects will be drawn from four different areas: the Wichita Standard Metropolitan Statistical Area; a county containing a medium sized city, a rural county with a high proportion of the aged; and a county which has experienced a high out-migration of the young and aged. Kansas was chosen because it is the only state which has an annual census. This will make the sample selection a relatively easly and inexpensive undertaking.

Significance to Biomedical Research and Program of the Institute: This study will add a much needed dimension to the understanding of factors affecting the migration of individuals, consequences of migration, as well as the conditions confronting aged persons in the population. It makes use of readily available data which reduces the cost of such a study.

Proposed Course: This research is scheduled to run for two years.

Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: Methods and Techniques for Using the 1960-1970 Census One Percent Public Use Samples

Contractor: National Data Use and Access Laboratories, Inc.
Money Allocated: $255,046

Objectives: The purpose of this proposal is to develop support techniques to facilitate the wider use of the 1970 Census Public Use Samples (PUS). The project emphasis will be on the handling of very large files by writing programs using COBOL and FORTRAN program to merge different types of records, create smaller work tapes, and provide programs for crosstabulations and analysis of data. The proposal also includes the writing of users' manuals and provision of classroom instruction in the use of these programs which will be available in the public domain at low cost.

Significance to Biomedical Research and Program of the Institute: The U.S. Census is an important source of data for demographers and other social scientists, as are other censuses which are made available from time to time. The sheer volume of the 1970 output, however, requires special effort. The needs of most researchers could be satisfied with select variables and records on a single manageable reel tape but the problem is to produce that reel or tape for individuals. DUALabs is a unique facility, incorporating previous experience with census, and several years' analysts lacking this experience.

Proposed Course: This project is scheduled to run for a period of two years.

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Behavioral Sciences Branch
Contract and Collaborative Research

Contract Title: The Social and Economic Variation in Marriage and Fertility Histories, 1967

Contractor: U. S. Bureau of the Census (reimbursable agreement)
Money Allocated: $23,000 (FY 1970)

Objectives: The purpose is to make available to the research community detailed tabulations of new and unique data from a large U. S. sample on the relationship between economic status, nuptiality, family breakdown and fertility. Relevant data collected in the Survey of Economic Opportunity of 1967 have never been analyzed.

Major Findings: (1) first births per 1,000 women in the ten-year period following marriage rose from 692 for white women marrying in the economic depression period 1930-1939 to 905 for those marrying in the period 1955-1959--and births came sooner after marriage for the latter cohort; (2) premarital first-birth rates also rose in this period; (3) age at first marriage is inversely related to lifetime fertility; (4) poor wives were younger at first marriage, had children sooner after marriage, and had larger families than other wives--their expectations of fewer future births accordingly may reflect a less realistic outlook; (5) wives who are not high school graduates expect more children than do wives with more education; (6) urban-rural fertility differentials have lessened, and birth expectations data for wives 30-39 years of age indicate a further narrowing in terms of the average number of children ever born; and (7) the data suggest that declines in the average size of completed families will occur in the near future and will continue, e.g., three-fourths of white wives 14-39 years of age who have already had two children expect no more children .

Significance to Biomedical Research and Program of the Institute: This project is highly relevant to the Institute's goal of better understanding the determinants of fertility in our society and particularly of the relationship between marital status and fertility in a time of rapid change.

Proposed Course: Findings were reported in "Previous and Prospective Fertility: 1967," Population Characteristics, Series P-20, No. 211, January 26, 1971, U. S. Bureau of the Census, and "Social and Economic Variations in Marriage, Divorce and Remarriage, 1967,"Current Population Reports, Series P-20, No. 223, October 7, 1971.

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Contraceptive Development Branch

The mission of the Contraceptive Development Branch (CDB) is the support of research programs which will lead to the development of an array of effective, safe, inexpensive, and reversible contraceptive methods acceptable to various population groups.

During the Fiscal Year 1972, the CDB has continued to support, through contracts, its dual approach toward the achievement of its major goal. The program of directed fundamental research into the human and animal reproductive processes continues. Its aim continues to be the clarification of gaps in the present knowledge of the reproductive processes, with the expectation that this fundamental knowledge will shed light on new contraceptive approaches. The product-oriented program which was initiated in FY 71 has been expanded to include a wider program of drug synthesis and testing and the continuation of the program dealing with contraceptive devices and drug delivery systems. An additional new phase of the product-oriented program is the establishment of a drug testing facility which will enable the CDB to rapidly and uniformly test compounds for antifertility activity. These activities are designed to enable the CDB to move swiftly in the short and long-range approaches to contraceptive technology within the next few years.

A. Directed Fundamental Research

Fundamental research supported by the CDB has focused on several areas which were considered promising for the development of new contraceptive methods. These areas represent both the female and male reproductive systems and, in many instances, these two are closely integrated.

The Oviduct

The importance of the oviduct in reproductive physiology cannot be overemphasized. It serves as the transport channel for both the female and male gametes and is the site where fertilization and the early development take place. The success or failure of the subsequent implantation is dependent upon the rate at which the oviduct transports the gametes and the fertilized ovum. The hormonal influences on the motility of the oviduct indicate dependence on both estrogens and progestins.[1] Observations on the oviduct have included direct visualization of the motility patterns, made with the help of force transducers implanted in this structure, as well as the observation of electrical activity of this organ under a variety of endocrine conditions.[2,3] It does appear that a pace-maker exists at the junction of the ampullary and isthmic portion of the oviduct and may control the overall activity of this structure.[4]

The degree of ciliation of the fimbriated end of the oviduct is hormone dependent and the number of ciliated cells determines the rate of ovum pickup following ovulation.[5,6] The presence of relatively few ciliated cells is detrimental to ovum pickup. The chemical nature of the oviductal secretion

in the rabbit is being investigated. The mucins elaborated in the separate segments of the oviduct are different.[7] Following ovulation, the quantity of the mucins increases dramatically without a change in the regional distribution.

Immunopharmacological characteristics of human oviducts are being investigated. Regional differences in dose-response curves of surviving tubes to histamine, serotonin, adrenalin, noradrenalin and acetylcholine have been observed. Tubal anaphylaxis is associated with increased rate and amplitude of tubal contractions as well as with increased activity of the tissue.[8]

The force transducers presently employed in monitoring oviductal function do not interfere with fertility.[2] Presumably, other transducers that are being developed for tracking the passage of the ovum along the oviduct, will not interfere with fertility.[9]

The Ovum and Blastocyst

The support of studies dealing with amino acid and nucleic acid metabolism of the developing rabbit blastocyst has been continued.[10] These studies are clarifying the regulation of early cell division. The transfer of macromolecules from the surrounding medium into ova and developing blastocysts is influenced by hormones and metabolic regulators.[11]

The requirements for in vitro maturation of mammalian ova, including human, have been described.[12,13,14] There appear to be considerable species differences in the ability of oocytes to undergo in vitro maturation. Similar situation exists in the ability of ova from different species to undergo in vitro fertilization. The morphology of the developing follicles has been described in the living macacque and squirrel monkeys. The vascularity of these structures is peculiar to the individual species.[14] The hormonal milieu surrounding the developing human oocyte is being ascertained. There appear to be changes in the content of various steroid hormones depending upon the size of the developing follicle.[15] The changes in hormones are being correlated with stages in oocyte maturation.

The Corpus Luteum (CL)

The corpus luteum is an ovarian structure formed following the rupture of the follicle, the primary function of which is to secrete the hormone progesterone which is necessary for the establishment and maintenance of pregnancy. In the human female, removal of the CL during the first seven weeks of pregnancy results in pregnancy failure.[4]

Interference with CL function can be accomplished in several ways. Research is being supported which relates to the inhibition of progesterone synthesis by the CL.[16] Another way to interfere with the action of progesterone is not through reducing its synthesis but by interfering with its action on the target organs, namely the oviduct and the uterus. This is being investigated under several contracts, the nature of which will be discussed under "Mechanism of Hormone Action." Methods for detecting ovulation and CL formation are being investigated.[9,16]

Prostaglandins (PG)

The Contraceptive Development Branch is supporting several researchers in the area of prostaglandin research. The important findings, emanating from these studies, indicate wide species differences with regard to the possible contraceptive utility of this group of substances. In the rhesus monkey, infusion of $PGF_{2\alpha}$ during the early luteal phase has no effect on the length of the menstrual cycle.[18] Infusion during late luteal phase shortens the cycle by one to two days. In the human female, infusion of $PGF_{2\alpha}$ appears to be without effect on either progesterone output by the CL or on the length of the menstrual cycle.[19] In the monkey, the effect on progesterone production appears to be biphasic, i.e. stimulation is obtained at low infusion rates and inhibition at high infusion rates.[20]

The presence of prostaglandins in the endometrium of the sheep and monkey has been confirmed. In the sheep the level of these substances in the endometrium increases toward the end of the luteal phase of the cycle and is considerably higher in the pregnant than in the nonpregnant animal. The physiological significance of these findings to CL function is presently being assessed.[21]

The mechanism of action of PG's on the uterine muscle is being investigated. Specific receptors for PG in the uterus have been identified.[18] A study of the localization of PG's in the female reproductive tract is being initiated as well as the study of the subcellular components involved in uterine contractility in response to PG.

The net result of these studies is the amassing of important biochemical and physiological information on the role of PG in reproductive biology. This information is utilized in the drug development program currently supported by the Branch.

Studies on Spermatozoa and Fertilization

During the past contract year, the research supported by contract funds has produced new important data along several important fronts. In the area of testis structure we are beginning to understand how substances that are found in the blood can be transferred into the tubules where spermatozoa are formed. This knowledge is important if one thinks in terms of delivery of special drugs to the testes and which could have an effect on sperm production.[22]

Important advances have been made in the area of the mechanisms of sperm motility. The propulsion of spermatozoa by means of their flagellae (tail) movements have been analyzed.[23,24] Similarly, the enzymes that may be important in controlling the beat of sperm tails are under study. The inhibition of these enzymes abolishes sperm motility and it is likely that the fertilizing potential of such spermatozoa is abolished.[24] This information may be of importance in devising specific spermicidal agents.

Advances have been made in the analysis of sperm enzymes that enable the spermatozoa to penetrate the cells surrounding the egg and the egg itself.[25,26] Inhibition of these enzymes by specific chemicals has produced infertile

spermatozoa. There exists presently a need for highly specific chemicals that would affect only the sperm enzymes and not similar enzymes found in other parts of the body. It has also been observed that in the female reproductive tract there are a number of naturally occurring inhibitors, the concentration of which appears to be under hormonal control.[26a]

Several researchers have produced antibodies against specific sperm enzymes and have observed that spermatozoa treated with these antibodies are infertile and that females injected with such antibodies have lowered fertility.[27,28]

Several interesting and important findings have been made in the area of epididymal physiology. It has been observed that the large amounts of fluids and hormones that are produced together with the spermatozoa are absorbed in the epididymis, whereas the spermatozoa remain in the epididymis tightly packed.[29] While the spermatozoa are in the epididymis they slowly acquire fertilizing ability.[30] This is dependent upon the presence of the male hormones called androgens. Withdrawal of androgens renders the spermatozoa infertile. This fact can be used for devising chemical antagonists that may interfere with epididymal function and render a state of infertility.

Studies on Hormone Action

Reproductive function is very closely associated with a number of hormones. Broadly categorized, the hormones fall into three general chemical classes, namely, steroids, polypeptides and proteins. The hypothesis currently held concerning the mechanism of hormone action is that the initial step in the expression of hormonal activity involves the interaction between the hormone and a specific receptor protein in the target tissue.

Receptors for the female sex hormones have been identified in the uterus and the oviduct.[31,32,33] Their physiochemical properties are being elucidated. Similar receptors have been identified in the hypothalamus and the pituitary.[34] Although the estrogen receptors in the various tissues appear to have similar characteristics their numbers appear to vary in the different target organs. Even within a single organ such as the oviduct there appear to be regional differences in the number of receptor sites.[31] Progesterone receptor sites in the uterus have also been identified. Specific rapid assays measuring the interaction between progestational drugs and the receptor system are operative.[32,33] These assays are facilitating the search for progesterone antagonists that could be useful in fertility control.

Studies are being conducted on the binding of LH and HCG in the ovary and the testes.[35] In order to conduct such studies large quantities of pure hormones are required. The preparation of pure HCG and LH and their respective subunits is being supported by funds provided by CDB.[36,37] These preparations are being made available to a large number of investigators.

Recent findings indicate that there may exist specific receptors for estrogens in the liver.[34] This is an important finding in view of the fact that the estrogenic component of the oral contraceptives is known to produce changes in liver function which in some patients can be severe enough to necessitate discontinuation of therapy.

B. Product-Oriented and Goal-Oriented CDB Programs

During FY 72, the Contraceptive Development Branch has continued to support programs involving drug development, including the testing of new compounds for antifertility activity, and development of techniques for permanent and reversible sterilization of both sexes.

1. Drug Development Program

Although there is a great necessity for new and safer contraceptive drugs, no such contraceptives have been introduced on the market during the past few years. Several new formulations involving reductions of the estrogen component, for the sole purpose of increasing the safety of the comination pill, have appeared on the market. One of the objectives of the CDB is to find new and effective chemical contraceptives which have fewer side effects than those presently being used by women. Another equally important aim is to search for a safe and effective contraceptive that men may use without the risk of loss of libido.

The drug program which began with support for nine contracts involving the synthesis and testing of antifertility agents has been in operation since June 1971. Some of these contracts involve the synthesis of new steroidal compounds which may be expected to show antifertility activity with lesser accompanying hormonal effects. The most interesting development from the structure-activity viewpoint comes from one study on silicon containing steroids.[38] It appears that the introduction of silicon into certain steroids markedly affects their biological response and causes a favorable separation of antifertility activity from other hormonal properties, e.g. estrogenic activity. The practical application of such a favorable change in the ratio of antifertility to estrogenic activity is somewhat distant, but an antifertility agent of greater potency than ethynylestradiol with lesser estrogenic effects would certainly be a desirable postcoital or antiovulatory contraceptive. In another study[39] involving the synthesis and testing of several metabolites of norethindrone and norethynodrel, none of the metabolites were found to display any favorable biological properties relative to the parent compounds. We are also supporting the synthesis of prostaglandin-like compounds.[40,41] Such drugs could act as luteolytic agents by interfering with corpus luteum function, or stimulate uterine activity and act as abortifacients, or induce menses at regular monthly intervals for use as a once-a-month pill. Unfortunately, the naturally occurring and most widely evaluated prostaglandins ($PGF_{2\alpha}$ and PGE_2) have a number of side effects which impose a limitation on their widespread utility in fertility regulation. Our synthetic prostaglandin program is still in its early stages, but emphasis has been placed on the synthesis and testing of pure chemical entities. In the past, because of the synthetic difficulties involved in preparing adequate quantities of material, many synthetic prostaglandin analogs were prepared as epimeric or racemic mixtures of compounds and tested as such, frequently leading to erroneous conclusions regarding their biological properties due to the agonist-antagonistic properties inherent in mixtures of closely related prostaglandin compounds. In one study[40] the synthesis of a variety of chemically pure prostaglandin analogs in sufficient quantity for testing their antifertility, luteolytic and antiprostaglandin properties has been completed.

Hopefully, some of the newly synthesized prostaglandins or their antagonists may prove to be useful without the liability of accompanying undesirable side effects.

Near the end of FY 72, new contracts were awarded (ten) encompassing unusual prostaglandin analogs, unique steroidal compounds and the preparation and testing of LH-RH and its analogs. One contract,[42] which involves the preparation of substantial amounts of LH-RH (the hypothalamic factor which controls the release of luteinizing hormone, the hormone which triggers ovulation), will provide us with the means to study the potential of LH-RH as an antifertility agent.

2. Testing Facility

During FY 72, the CDB has taken the important step of setting up a testing facility for the evaluation of compounds for contraceptive activity in both the male and the female. The Branch has identified a contractor that has the ability to undertake screening and advanced evaluation of compounds. These compounds will come from the synthetic program presently supported by the Branch. It is hoped to expand the program to screening of selected chemicals from Government, academic and industrial sources. The uniform screening program will permit direct comparisons of activities among a large number of compounds which are presently difficult to assess.

3. Development of Sterilization Techniques

This program was initiated in FY 71 and has as its primary aim the development of reversible sterilization techniques in both the male and the female. In the male reversible sterilization program, a variety of devices are being evaluated which would render vasectomy easily reversible.[43,44,45] During the past year, advances have been made in the study of device implantation techniques, interaction between the device and the surrounding vasal tissue, improvement of device structure and the actual efficacy of the implanted devices. Present studies are all being done in a variety of laboratory animals and only after they prove to be safe and efficacious will the Branch consider the support of human clinical trials.

Reversible female sterilization by occlusion of the oviducts is a much more complicated procedure than the occlusion of the vas.[46,47] Introduction of a variety of tubal plugs through the tubo-uterine junction is complicated by the difficulty in visualizing the tubal opening into the uterus. Research supported by CDB funds has lead to the development of a steerable hysteroscope which will make the visualization of the tubo-uterine junction possible.[46] In conjunction with the development of this prototype instrument, studies are underway which concern themselves with fabrication of plugs which can produce total occlusion of the oviduct and prevent spermatozoa from reaching the fertilization site. It is anticipated that the work on the female reversible sterilization system will be more time consuming than that for the male. However, it does offer the potential of affecting sterilization without resorting to abdominal surgery.

C. CDB Activity in the Area of Hormone Distribution

At the present time the Branch, in cooperation with the National Pituitary Agency, is supplying investigators with uniform purified batches of HCG and its subunits.[36] It is proposed to expand this program to include ovine LH and its subunits[37] and also LH-RH.[42]

REFERENCES

1. Blandau, R. J., 70-2141, $167,734. An interdisciplinary project on the biology of the oviduct and gamete transport. University of Washington

2. Fromm, E., 69-2189, $64,893. A bioengineering approach to the study of tubal activity in contraception. Drexel Institute

3. Nelsen, T., 70-2257, $117,000. Physiology of the oviduct. Stanford University

4. Csapo, A., 69-2194, $56,342. The action of estrogens and progesterone upon uterine function in pregnancy. Washington University

5. Odor, D. Louise, 70-2142, $14,402. Ultrastructural studies on the oviduct of intact normal, the ovariectomized and ovariectomized-hormone treated rabbit and pig-tailed monkey, Macaca memestrina. Medical College of Virginia.

6. Brenner, R. M., 70-2146, $11,390. Control of ciliogenesis in the primate oviduct. University of Oregon

7. Leppi, T. J., 70-2311, $19,297. Morphochemical analysis of mucins in the mammalian oviduct. University of New Mexico

8. Feigen, G. A., 70-2157, $79,807. Immunopharmacology of the human fallopian tube. Stanford University

9. Stegall, H. F., 72-9-067, $45,600. Detection of ovulation in chronically instrumented animals. University of Washington

10. Hahn, W., 70-2259, $26,192. The effect of progesterone and estradiol on RNA synthesis in the rabbit oviduct and its relationship to early embryonic development. University of Colorado

11. Glass, L., 70-2309, $34,804. Transfer of maternal macromolecules to mammalian eggs. University of California

12. Soupart, P., 70-2162, $44,278. Sperm capacitation as a target for contraception. Vanderbilt University

13. Zamboni, L., 69-2220, $45,978. Morpho-physiologic studies on mammalian gametes prior to and during fertilization in vivo and in vitro. Harbor General Hospital

14. Dukelow, W. R., 70-2061, $28,914. Control of ovulation and capacitation in the nonhuman primate. Michigan State University

15. Taymor, M., 71-2225, $23,800. Endocrine control of human oocyte maturation. Peter Bent Brigham Hospital

References, cont'd

16. Salhanick, H., 70-2319, $45,500. Development of *in vitro* screening procedures for inhibitors of progesterone synthesis. Harvard University

17. Yon, E. T., 72-9-023, $123,026 (2 years). Development of techniques for study of oviductal transport and detection of ovulation in primates. Case Western Reserve University

18. Kirton, K. T., 69-2208, $72,903. Luteolysis as an approach to contraception. Upjohn Company

19. LeMaire, W. J., 71-2238, $115,909 (2 years). Effects of prostaglandins on ovarian function. University of Miami

20. Speroff, L., 71-2118, $25,470. The role of prostaglandins in ovarian physiology. Yale University

21. Inskeep, E. K., 69-2215, $39,710. Relationships of endometrial prostaglandins and luteal function. West Virginia University

22. Fawcett, D. W., 69-2107, $67,682. Morphological studies on spermatogenesis, sperm maturation, and means of inducing reversible infertility in the male. Harvard University

23. Rikmenspoel, R., 70-2156, $38,170. Control mechanism of sperm motility. State University of New York

24. Nelson, L., 70-2313, $45,644. Neurochemical control of sperm motility. Medical College of Ohio at Toledo.

25. Srivastava, P., 70-2147, $45,835. Enzymes of the sperm acrosome, occurrence of the inhibitors of these enzymes in seminal plasma and the role of the enzymes and inhibitors in capacitation of sperm and in the penetration and fertilization of ova. University of Georgia

26. Stambaugh, R., 70-2161, $36,893. Studies on acrosomal enzymes effecting penetration of the zona pellucida. University of Pennsylvania

26a. Schumacher, G.F.B., 70-2317, $24,122. Protease inhibitors in human genital secretions. University of Chicago.

27. Metz, C. B., 70-2253, $83,025. Immunochemical investigation of sperm transport and fertilization in the mammal. University of Miami

28. Goldberg, E., 69-2162, $45,225. Biochemical studies on spermatogenesis and spermatozoa. Northwestern University

29. Amann, R., 69-2137, $65,359. Role of the epididymis in sperm maturation. Pennsylvania State University

References, cont'd

30. Orgebin-Crist, M., 69-2166, $50,294. Hormonal control of sperm maturation. Vanderbilt University

31. Jensen, E.V., 69-2108, $31,944. Estrogen receptor substances of oviduct tissue. University of Chicago

32. Toft, D., 70-2165, $47,941. Studies on steroid hormone receptors and their role in reproductive biology. Vanderbilt University

33. Bardin, C. W., 71-2228, $20,694. Assay of progesterone receptor activity in the guinea pig uterus. Milton S. Hershey Medical Center

34. Eisenfeld, A. J., 70-2258, $58,500. Estrogen binding in reproductive organs. Yale University

35. Ryan, R., 71-2232, $38,446. A model for studying inhibition of LH action on the corpus luteum. Mayo Clinic and Foundation

36. Canfield, R. E., 70-2251, $83,044. Pilot study for the large scale preparation of purified human chorionic gonadotropin. Columbia University

37. Ward, D., 69-2221, $119,340 (3 years). Physiological aspect of the attachment of carbohydrate to luteinizing hormone. University of Texas

38. Skinner, W., 71-2462, $108,908 (1 year). The synthesis and testing of antifertility compounds. Stanford Research Institute

39. Wall, M.E., 71-2457, $112,855 (1 year). Synthesize, furnish and test antifertility compounds. Research Triangle Institute

40. Fried, J., 71-2117, $67,618 (15 months). Synthesis of prostaglandin analogs for biological studies.

41. Revesz, C., 71-2463, $180,640 (2 years). Synthesis and antifertility testing of prostanoic acid derivatives. Ayerst Research Laboratories

42. White, W. F., 72-2722, $125,300 (1 year). Synthesis and testing of gonadotropin releasing hormone (Gn-RH) to establish antifertility and other endocrine activity.

43. Brueschke, E., 71-2229, $148,584. Design and development of implantable contraceptive devices for use in the male. IIT Research Institute

44. Nuwayser, E., 71-2227, $344,847 (2 years). Development of new sterilization techniques. Abcor, Inc.

45. Freund, M., 71-2223, $65,000. The use of Bionyx control as a contraceptive (reversible vasectomy device) in men. New York Medical College

References, cont'd

46. Brueschke, E., 71-2233, $252,992. Development of an intrauterine sterilization system for the female. IIT Research Institute

47. Erb, R. A., 71-2237, $85,967. Device and technique for blocking fallopian tubes. Franklin Institute

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NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Symposium on Biology of Corpus Luteum
Contractor: Society for the Study of Reproduction
c/o University of Kansas Medical Center
Money Allocated: $6,000 (FY 72)

Objectives: To present the subject of the biology of the corpus luteum to reproductive physiologists with varied backgrounds and from different disciplines and hence to touch many areas; to inform them of the present status of the field, provide new research data, outline areas of future research, and to examine the potential role of the corpus luteum as an object for the development of new contraceptives.

Significance to Biomedical Research and Program of the Institute: Research on the corpus luteum has been identified by the CPR to be a priority field both by the contract and grant routes, and periodic assessment of progress from time to time, along with the exploration of new avenues of attack, are considered imperative if new ground is to be broken and further progress anticipated from both experienced and new investigators.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Estrogen-Receptor Substances of Oviduct Tissue
Contractor: University of Chicago
Money Allocated: $45,413 (FY 69 Funds), $29,815 (FY 70 Funds), $31,944 (FY 71 Funds), $36,265 (FY 72 Funds)

Objectives: The investigators are studying the molecular mechanism of estrogen activity in oviduct tissue by biochemical and autoradiographic determination of the presence, intracellular localization, and chemical nature of specific "estrogen receptor" proteins.

Major Findings: The uptake and retention of tritiated estradiol by calf oviduct *in vivo* appears qualitatively similar to that of calf and rat uterus. It is sensitive to the same specific inhibitors (Upjohn 11,100, Park-Davis CI-628) and to sulfhydryl reagents and is not blocked by actinomycin-D, puromycin or cycloheximide.

Quantitatively, oviduct in both the calf and the rat shows a lower uptake and binding of estradiol per unit tissue weight than does uterus. In most *in vivo* experiments with the hypophysectomized or ovariectomized rat, the estradiol uptake at various times after hormone injection was highest in uterus, about half this amount in ampulla and isthmus and somewhat lower in vagina; in a few experiments the vagina level was about the same as that of ampulla and isthmus. In the rat it was not possible to obtain sufficient fimbria for dependable measurement. At 37° *in vitro*, calf ampulla usually bound slightly more estradiol than did fimbria, and isthmus bound considerably less; in some experiments the levels in ampulla and fimbria were comparable.

With calf tissues, there is evidence which suggests that the binding affinity of the receptor protein in the oviduct cytosol may not be as strong as that in the uterus. The cytosol from fimbria contains fewer receptors than cytosol from isthmus and the ampulla. However, the receptor system in fimbria may function in an especially efficient manner

Considerable progress has also been made in the area of purification of the estradiol nuclear receptor from the calf uterus. Microgram amounts of the 5S nuclear complex, as well as the 4S binding unit of the cytosol complex have been isolated in essentially pure form.

Significance to Biomedical Research and Program of the Institute: This particular study is an important step in understanding hormone and possibly anti-hormone action at the cellular level and is directly relevant to the purposes of the Contraceptive Development Program.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Neural Control of the Mammalian Oviduct
Contractor: University of Massachusetts
Money Allocated: $14,955 (FY 69 Funds), $14,840 (FY 70 Funds), $13,850 (FY 71 Funds)

Objectives: Using rabbits in which the oviducts have been denervated by stripping vaginal and recto-vaginal septal fascia and removing the inferior mesenteric ganglion, spermatozoa and ova transport are being studied both in the denervated state and in the presence of autonomic drugs. An initial stage of the program is involved with establishing the effectiveness of the denervation procedure, using the disappearance of norepinephrine from the oviduct as an index. Rabbits undergoing this procedure are mated after 10 days. In order to determine the migration rate of spermatozoa, the tubo-uterine junction of one side is ligated at 1, 2, 3, or 4 hours post coitum. Forty-eight hours later, the animals are killed and the oviduct flushed to recover ova for study by phase microscopy for evidence of fertilization.

Ovum transport is also being studied in rabbits mated 10 days after the denervation procedure. Animals are killed 24, 36, 48, 60, 72, and 84 hours post coitum and laparotomy immediately performed. The viscera are rapidly frozen to stop oviduct motility and, after thawing, the oviducts are cleared in order to determine the exact position of each ovum.

Major Findings: Epinephrine increased the rate of ovum transport over controls at 60 and 70 hours post coitum ($p<0.01$); 50% of the rabbits in the epinephrine experiment had some ova in the uterus at 60 hours after mating.

Phenoxybenzamine, when compared to controls, inhibited ovum transport at 48 hours post coitum, but was not significantly different at any other time periods tested. Propanolol did not significantly alter the mean rate of transport.

Perfusion of 6-hydroxytopamine through the oviduct for the purpose of inducing chemical sympathectomy resulted in significant depression in tissue norepinephrine levels.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to elucidate the role of the oviduct in gamete transport.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: A Bioengineering Approach to the Study of Tubal Activity and Contraception
Contractor: Drexel Institute of Technology
Money Allocated: $116,456 (FY 69 Funds), $38,437 (FY 70 Funds), $7,688 (FY 71 Funds), $64,893 (FY 72 Funds)

Objectives: The objective of the contract is to study tubal contractile activity in the unrestrained animal with respect to the various ovarian cycle phases, and to assess the role such activity plays with regard to ovum transport. The investigator has concentrated on the development of the appropriate transducers and telemetric packages, with initial attempts at animal implantation.

Major Findings: Further design and testing improvement of the reduced size (.032" O.D.) silastic element transducer have been accomplished. Improvements in design include a more reliable and repeatable contact system, the addition of a flexible Estar "beam" to add directional preference and increased sensitivity to the sensing element as well as the use of a silastic tubing sheath to increase flexibility and further approach an isotonic device.

These improvements in transducer fabrication have provided a more sensitive device which has a preferred operating direction, and is relatively insensitive to forces applied in a perpendicular direction. Forces applied to cause tension in the sensing element cause increases in sensor resistance while those forces which result in compression cause decreases in sensor resistance. Implantation of these transducers at the tubo-uterine junction of rabbits had no deleterious effect on subsequent fertility. A new silicon strain gauge transducer has been manufactured and is presently undergoing physical testing prior to its implantation in monkeys.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: A Biochemical Investigation of the Oviduct-Uterus System in the Pseudopregnant and Normal Cycling Hamster
Contractor: Worcester Foundation for Experimental Biology
Money Allocated: $37,182 (FY 69 Funds) for two years, $9,347 (FY 71 Funds)

Objectives: The objective of this research project is to determine whether there is a regulatory relationship between oviduct and ovary by examining "certain biochemical parameters in the oviduct-uterus system throughout normal estrous cycles and pseudopregnancy, and 'determining' the effects on these parameters of various experimental manipulations." This is being done in the hamster which has certain advantages for the study of ovarian regulation. At different stages in the ovarian cycle, oviduct and/or uterus are removed and the ability of these tissues to incorporate labeled precursors into RNA, protein and lipid in vitro measured. The effect of removing the oviduct or uterus on the ovarian cycle is being studied to determine whether there are luteolytic factors arising in the oviducts, or arising in the uterus but mediated through the oviduct. Similar experiments will be done in the pseudopregnant animal.

Major Findings: The incorporation of precursors into total protein lipids and RNA has been studied in hamster oviducts and uteri. Oviduct removal does not cause significant differences in the biochemical parameters in the remaining uteri. Hysterectomy, however, results in a reduction of protein synthesis in the oviduct on all days except day 1 of the cycle. There is an increase in lipid synthesis on all days except day 2 of the cycle.

Neither oviduct removal nor hysterectomy appears to alter the type of vaginal discharges or the length of the hamster estrous cycle.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research devoted to identifying the factors responsible for corpus luteum formation, function, and regression.

Proposed Course: Terminated

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Factors Influencing Mammalian Oviductal Secretions
Contractor: Johns Hopkins University
Money Allocated: $32,090 (FY 69 Funds), $41,362 (FY 70 Funds), $42,686 (FY 71 Funds), $46,743 (FY 72 Funds)

Objectives: The objectives of this research project is to continue the study of the electrical potential difference exhibited across the serosal and mucosal surface of the rabbit oviduct as related to net ion transport. Preliminarily, they have found a net chloride transport across the membrane under short-circuited conditions. Since the net chloride flux is equivalent to the short-circuited current, it has been reasoned that the electrical phenomena can be accounted for by the active transport of chloride. They are now developing an in vitro preparation for the primate oviduct similar to that described for the rabbit, in order to extend their studies of the active transport of ions using the short-circuit technique to this species. Lastly, they wish to determine the effects of drugs and hormones on active transport in the in vitro oviduct system using this as a screening technique for potential contraceptive drugs.

Major Findings: Isolated preparations of oviducts from rabbit, rhesus monkey and humans have been developed and utilized to study the secretory processes of these tissues. The electrical activity of these tissues are dependent on energy derived from oxidative metabolism. Isotopic flux studies showed that the isolated oviducts activity transport chloride and sodium and that these active transport processes are influenced by a variety of drugs. Isoproterenol, a beta-adrenergic agent, stimulated the active transport of chloride into the lumen of the oviduct and free copper ions stimulated active transport of sodium out of the oviduct. Prostaglandins affect the electrical parameters greatly, but their affect on ion transport has not yet been determined. Prostaglandins E_1 and E_2, as well as beta-adrenergic agents, accelerated the rate of fluid secretion by everted oviducts. Both of these compounds stimulated adenyl cyclase activity of oviductal epithelial cells, which indicates a central role for cyclic AMP in oviductal secretion.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the mechanisms responsible for the secretion of oviductal fluids and the influence of these fluids on gamete transport and survival.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: An Interdisciplinary Program Project on the Biology of the Oviduct and Gamete Transport
Contractor: University of Washington School of Medicine
Money Allocated: $192,490 (FY 70 Funds), $177,734 (FY 71 Funds), $177,734 (FY 72 Funds)

Objectives: This is a comprehensive interdisciplinary program project on the basic biology, physiology, and endocrinology, of oviductal function in rabbits and monkeys. Seven different specific aims are enumerated: 1) the development of techniques for direct observation of the normal function of the various segments of the oviduct in situ; 2) development of miniature sensing devices and telemetry equipment that will permit study of the normal activity of the oviduct in unanesthetized, unrestrained, rabbits and monkeys; 3) study the electrophysiological characteristics of oviductal musculature; 4) investigation of intrinsic innervation, pharmacological responses, and the possible role of pacemakers in oviduct function: 5) ultrastructural studies of the epithelium and musculature under different physiological conditions; 6) development of techniques of in vitro cultivation of oviductal tissue as a potential bioassay system; and 7) studies of the mechanism of sperm transport through the oviducts.

Major Findings: Studies on ovum transport in the rabbit have established that the passage through the ampullary section of the oviduct is slowed down by estrogen and stimulated by estrogen withdrawal. An antiestrogen given in conjunction with estrogen speeds up the ampullary transport. The transport of ova through this region is correlated with direct observation of oviductal muscle contractility. The effects of the estrogen withdrawal have been evaluated. After ovariectomy the rate of ovum transport is speeded up significantly. The work that has been carried out in rabbits is going to be substantiated by work with monkeys that are presently being conditioned.

The study of particle movement by ciliary beat in the lower part of the isthmus is directed toward the ovarian end of the oviduct. The uterotubal junction exhibits strong ciliary beat towards the uterine lumen. As one proceeds up the oviduct, there is a fairly clear change in direction of ciliary beat. In the study of proteolytic enzyme activity of spermatozoa it was observed that the activity is present in developing spermatids. The proteolytic activity may be of importance in the penetration of cervical mucus.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the various functions of the oviduct which are responsible for gamete transport and survival, fertilization, and transport of the fertilized egg to the uterus.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Ultrastructural Studies on the Oviduct of the Intact Normal, the Ovariectomized and Ovariectomized-Hormone Treated Rabbit and Pig-Tailed Monkey, Macaca-Nemestrina
Contractor: Medical College of Virginia
Money Allocated: $13,195 (FY 70), $12,764 (FY 71), $14,402 (FY 72)

Objectives: This research project is part of an interdisciplinary research program on oviduct structure and function being carried out at the University of Washington. This investigator will carry out at the University of Virginia certain morphological aspects of the work on specimens obtained in Seattle where correlative in vivo observations and physiological measurements are being made on the same material.

A systematic study of the ultrastructure of the fimbria, ampullae, and isthmus of the normal rabbit oviduct in the pre- and post-ovulatory period, in the ovariectomized animal, and in ovariectomized animals receiving estrogens and progesterone singly and in combination will be made. Studies will not be confined to the epithelium but will include fine structural observations on the smooth muscle which may be correlated with changes in oviduct motility. With this base line established on rabbits, similar studies will be made on the oviduct of the pig-tailed macaque.

Major Findings: When long-termed (16-37 weeks) ovariectomized rabbits are treated with 10 μg of estradiol-17β per day for intervals of 1 through 10 days, changes occur in the epithelium of the oviductal fimbriae. After 2 through 6 days of treatment there is a significant reduction in the percentage of ciliated as compared to non-ciliated cells in most, but not all, of the animals. A hypertrophy of, and loss of cilia from, many of the ciliated cells occurs. Subsequently stages in ciliogenesis are observed. In animals where the percentage of ciliated cells falls below 40, egg masses placed on the fimbrial surface under in vivo conditions are not moved. When 40-50% of the cells are ciliated, slow ineffective transport occurs. Reciliation is almost complete by 7 days of treatment. Transport of eggs to or through the ostium is quite effective on days 7, 9 and 10, but is not as rapid as in normal post-ovulatory controls. In untreated ovariectomized rabbits the fimbrial epithelium has an average of 44% ciliated cells and egg masses are transported through the ostium slowly in 25% of the trials. Secretory cells hypertrophy; have first an increased number of free ribosomes and then a granular endoplasmic reticulum profiles. The amount of secretory material in the cells varies considerably from animal to animal, even when treated for the same number of days. It does not appear to play a role in transport of eggs across the fimbrial surface.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of

the Contraceptive Development Branch to support studies of the ultrastructure of the mammalian oviduct and its responses to changes in the hormonal environment.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Measurement of Blood Flow in the Mammalian Oviduct
Contractor: Washington State University
Money Allocated: $14,065 (FY 70), $14,220 (FY 71)

Objectives: This research project is designed to measure blood flow to the mammalian oviduct and to describe the structural geometry of the oviductal vasculature. For the latter objective, silicone rubber elastomer will be injected, and casts prepared in the Anatomy Department and studied grossly with the dissecting microscope, and microscopically, with 100 to 150 micron sections. For measurement of blood flow volume, two methods are proposed: (1) indicator-diffusion using hydrogen gas; and (2) thermoconductivity. In the first method, blood flow is related to the rate of removal of hydrogen from the tissue, while in the second, the rate of dissipation of heat is the variable related to flow. These methods have been selected because they offer the best opportunity to estimate capillary flow, and appear to be adaptable for use in unanesthetized animals. Two transducer configurations will be tested: (1) a needle type, with the measuring element inserted into the tissue; and (2) a cuff type in which the measuring device is in contact with the surface of the oviduct. During acute experiments the 4-amino-antipyrene indicator-diffusion technique will be utilized for comparison purposes in the measurement of uterine blood flow. This technique will be modified using radioiodinated antipyrene when the oviduct comparison is made. Eventually, it is planned to study the effects of sex hormones on oviductal blood flow in the oophorectomized animal.

Major Findings: In the anatomical studies the major thrust during this period was to obtain differential injection of arteries and veins with media of two different colors and to use the two-color technique to differentially inject the ovarian utero-ovarian circulation.

It was found that one remaining artery, either ovarian or utero-ovarian, was capable of supplying the entire reproductive tract on both sides. This finding, supported by the results of single and double ligation trials, indicates the presence of ovarian-utero-ovarian as well as side-to-side anastomoses. The presence of such anastomoses, suggested by the above evidence, was confirmed by careful microscopic (dissecting microscope) tracing of the vasculature.

While there appears to be free anatomotic communication between the ovarian and utero-ovarian circulations, double dye injections into the intact circulation indicates that the ovarian artery supplies the ovary, oviducts and uterotubal junction, the utero-ovarian supplies the uterine horns, and the two circulations overlap near the cranial extremity of the uterine horns..

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of

the Contraceptive Development Branch to support studies on the vascular supply and blood flow patterns in the mammalian oviduct.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Control of Ciliogenesis and Secretory Cell Growth in the Primate Oviduct
Contractor: Oregon Regional Primate Research Center
Money Allocated: $14,831 (FY 70), $11,390 (FY 71)

Objectives: The primary objective of this research project is to use this system for fundamental studies of how hormones regulate cell differentiation in the oviduct. Autoradiographic studies on amino acid uptake and protein synthesis will be carried out with *in vivo* and *in vitro* administration of labeled precursors. It is hoped thus to determine sites of synthesis of microtubal protein and the mode of growth of ciliary shafts. By use of tritiated thymidine and tritiated uridine applied at critical periods in the process of ciliogenesis, the investigator hopes to shed light on the controversial question of the presence of DNA in basal bodies and of RNA in basal body precursors.

Major Findings: Organ culture of oviducts from infant monkeys injected with estradiol benzoate indicates that protein synthesis is no longer taking place in those cells which are completing basal body formation and cilia growth. Colchisine inhibits basal body formation in the *in vitro* system without apparent damage to other cell organelles.

A technique has been developed to gently disrupt the ciliated cells of the oviduct and release as a unit the cortical region of the cell containing the ordered rows of basal bodies and cilia.

Organ culture of fetal monkey oviducts obtained at 100 days of gestation has shown that control 100-day oviducts did not make cilia over a 5-day culture period. However, those exposed to estradiol in culture medium did make cilia. These were the first oviducts to ever show a complete estrogen response *in vitro*.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the formation and hormonal dependence of oviductal cilia and the role these cilia play in gamete transport.

Proposed Course: Renewal not requested.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Immunopharamacology of the Human Fallopian Tube
Contractor: Stanford University
Money Allocated: $40,793, (FY 70), $79,881 (FY 71), $91,602 (FY 72)

Objectives: The work to be carried out under this contract involves two aspects of oviductal physiology: (1) characterization of the permeability and transport characteristics of oviductal epithelium; and (2) characterization and quantitation of the oviductal response to the anaphylactic reaction between an antigen and the specific antibody bound to oviductal cells. Work will be carried out in laboratory animal and human oviductal tissue, in vitro.

Major Findings: Studies have been made on the immunopharmacological properties of human tubes obtained from approximately 90 donors. Work during the first year of operation established regional differences in dose-response curves of surviving tubes to histamine, serotonin, adrenaline noradrenaline, and acetycholine. Positive, functional, evidence of anaphylaxis, expressed as an increase in rate and amplitude of tubal contraction, was obtained in experiments involving direct passive sensitization with sera of human subjects having documented allergies to ascaris and aspergillus. Ampullar tissues degraded histamine at twice the rate found for the isthmus. The process of reversed passive sensitization was studied this year to obtain quantitative data concerning the relationship between the load of sensitizing agents, the dose of challenging antibody and the release of vasoactive materials. Typical optimum proportion functions were obtained by sensitizing tissues with pooled human globulins and challenging them with rabbit antibodies to human gamma-globulins or goat antibodies to human IgE. A comparative study was made of the various human globulin preparations in this connection. The electrophysiological aspects of anaphylaxis, studied by means of microelectrodes inserted into single cells, showed that effective challenge results in a burst of high frequency spikes over the usual slow potential-carrier wave. A study of the conditions determining the liberation of SRS-A from tissues was initiated as well as studies dealing with the effects of antibody concentration and temperature on the kinetics of tubal sensitization.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support innovative studies on the physiological and pharmacological properties of laboratory animal and human oviductal tissue.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Effects of Steroid Hormones on RNA and Protein Synthesis in the Mammalian Oviduct
Contractor: The University of Texas at Austin
Money Allocated: $40,459 (FY 70), $21,700 (FY 71)

Objectives: The objective of this research project is to study RNA and protein synthesis in the mouse, rabbit, and guinea pig oviduct as a function of stage in the estrous cycle, time after hormone treatment, or sequence of hormone treatment in the ovariectomized animal. If initial findings indicate that there is evidence of hormonal control of genetic transcription and translation in the oviduct, the investigators will proceed with a more precise type of documentation of such hormonal control, including studies of the various anatomical regions of the oviduct. In vitro studies of whole nuclei and of template activity in vitro of isolated and purified chromatin with added RNA polymerase are also planned. It is hoped to be able to isolate and characterize oviductal polyribosomes and to analyze their amino acid incorporation activity in vitro. All these responses will be measured after different hormone treatment in spayed animals or at different stages of the estrous cycle in intact animals. Eventually, the investigators plan to seek further information by techniques of RNA-DNA hybridization and competition experiments.

Major Findings: The data indicates that in the mouse oviduct the content of protein, RNA are highest during proestrus. The amount of DNA does not tend to fluctuate to any great extent during the cycle. Both RNA and protein content of the oviduct are primarily under estrogenic control and progesterone may exert a suppressive effect. Unlike the situation in the uterus, progesterone did not antagonize the increase in oviductal weight produced by estradiol. Mating has little effect on oviductal biochemistry during blastocyst transport. Part of the work described above has been accepted for publication.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the synthetic capabilities of the mammalian oviduct and their influence by hormonal environment.

Proposed Course: Terminated

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Studies on Steroid Hormone Receptors and Their Role in Reproductive Biology
Contractor: Vanderbilt University
Money Allocated: $40,088 (FY 70), $47,941 (FY 71), $49,908 (FY 72)

Objectives: The objectives of this research project are: (1) to study further the already established interaction of estrogens with receptor substances of rat uterus in an effort to elucidate the role of the receptors in hormone action; and (2) to investigate estrogen and progesterone localization and binding in chick oviduct, a tissue which recently has been reported to contain a specific binding protein for progesterone.

Major Findings: Both the estrogen receptor from uteri and the progesterone receptor from chick oviducts can bind to purified DNA. This reaction has been partially characterized. The binding is relatively strong and could be involved in the in vivo attachment of receptors to chromatin. Physiological significance of this binding is yet to be explored. Specific progesterone binding proteins (receptors) have been identified and partially characterized from mouse and rat uteri. These proteins are quite similar to the progesterone receptor of the chick oviduct; however, some minor differences are apparent. Preliminary studies indicate the presence of similar binding proteins in guinea pig and human uteri. Estrogen treatment increases the concentration of progesterone receptors in the chick oviduct and also appears to alter the receptor molecules in a qualitative manner. This latter effect is evident by a change in the sedimentation properties of receptors following estrogen treatment.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the steroid binding substances in reproductive tract tissues.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Physiology of the Oviduct
Contractor: Stanford University Medical School
Money Allocated: $91,585 (FY 70), $10,000 (FY 71) $141,864 (FY 72)

Objectives: The objective of this investigation is to develop electrodes, transducers and other instrumentation for study of electrical, mechanical, pressure, and flow phenomena and to use these instruments to investigate the functional properties of the muscle coat of the oviduct (1) as small segments in the tissue bath, (2) an isolated organs and (3) in the intact subject. By determining changes in properties of the oviductal muscles as a function of hormonal environment, knowledge will be sought as to the mechanisms which control oviduct function.

These studies will involve measurements of the electrical and contractile behavior of oviductal tissue using excised oviducts of rabbit and man, at various stages of the reproductive cycle. Studies of pacemaker activity, the interrelationship of electrical activity from one area to another, correlation of electrical and mechanical activities, and intraluminal pressure changes will be conducted. The final phases of the study will involve the measurement of these functions in intact, free-roaming animals, and hopefully, eventually in women. All data will be programmed for computer analysis and a mathematical model of the system will be undertaken similar to one the investigator has developed for intestinal smooth muscle.

Major Findings: The aim of this study is determination of the electrical and mechanical activity of mammalian oviduct *in situ*. To accomplish this goal fabrication of a variety of transducers has been undertaken. The electrical transducer (electrode) consisting of a glass encapsulated platinum wire has been fabricated and tested both *in vitro* and *in vivo*. The *in vitro* data obtained with this electrode correlate well with the sucrose gap studies. These electrodes are small enough that several of them can be attached in tandem to a single oviduct. The problem of external leads has not been completely resolved.

A new force transducer has been fabricated at the integrated circuit laboratory. This transducer is small enough to be attached to the rabbit oviduct. They represent silicon chips on which integrated circuits are photo-engraved. Preliminary data indicate that these units are functioning well. Problems of encapsulation and lead wires are not completely solved.

The pressure transducer of approximately 0.6 mm in diameter has been fabricated and tested. This sensor is mounted at the tip of the catheter and responds to pressure variations.

There is good indication that because of the advanced technology both the force and pressure transducers can be produced in quantity.

Computer programs have been written for the analysis of data emanating from the in vitro and in vivo studies with electrodes and transducers.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to encourage scientists from other disciplines to apply their unique skills to studies of reproductive function.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Oviduct Cilia: Structure, Function, Development
Contractor: University of California
Money Allocated: $34,343 (FY 70), $9,097 (FY 71), $35,038 (FY 72)

Objectives: The stroke form and fine structure of oviductal cilia is to be explored using techniques previously applied to mussel gill cilia. The aim is to provide information on the beat mechanism of oviductal cilia and to evaluate the influence of various agents on the mechanism. Techniques to be employed in these studies include high-speed cinematography, electron microscopy, and serial-section analysis of stroke form and top structure. An attempt will be made to integrate this information into a unified theory of ciliary beat based on the interaction of various ciliary organelles.

Major Findings: The ultrastructure of mouse oviductal cilia conforms to the general pattern. It is likely that mechanism of cilial motility is similar to motility of other organelles (flagella). The investigators have made some original observations using new techniques. These observations are as follows:

a. In squash preps ciliary beat can be seen. The cilia are short and well-defined metachromism is not evident.

b. Freeze-fracture replicas of oviduct cilia along the ciliary membrane demonstrate a smooth appearance with a few particles scattered at random, while cross-sectional fractures produce a resemblance to the nine-fold symmetry characteristic for cilia. For oviduct cilia, they have discovered the "ciliary necklace" below the basal plate. The "necklace" is a good candidate for the energy transducing system necessary for cilial beat.

c. With electron scanning microscopy and special preparative techniques they have assessed the relationship between bands of cilia in the fimbria. It appears that long bands of cilia beat at angles to each other. Adjacent bands of cilia point in opposite directions, perhaps producing the counter-current eddies necessary for sucking in the ovum after ovulation. The fimbriated end appears as a thick carpet solidly encrusted with cilia. In the lower portion of the oviduct, cilia are distinct, and separate clumps arise in crevice-like protrusions.

Transmission electron microscopy of ciliary tips indicates the presence of a corona of very fine fibers.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the mechanism of action of oviductal cilia and their role in gamete transport.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Experimental Methods of Interrupting Pregnancy
Contractor: Jefferson Medical College
Money Allocated: $37,660 (FY 70), $44,220 (FY 71), $49,322 (FY 72)

Objectives: The investigator will investigate, in rats, two non-surgical methods for inducing abortion. The first involves microwave electromagnetic irradiation of the pregnant uterus to elevate the temperature of the fetus; the second involves the combined use of microwave electromagnetic irradiation and certain cancer chemotherapeutic agents to eliminate the fetus, without injury to the mother.

Major Findings: The investigators have developed methods for modifying commercial microwave ovens for better control and direction of radiation, designed and constructed antennas for the direction of energy, and studied large numbers of pregnant rats exposed to microwave radiation. They have concluded that the feticidal effects of microwave irradiation of exteriorized gravid uteri is primarily due to localized temperature elevations. Rats exposed to feticidal doses of microwave radiation exhibited subsequent fertility. Embryonic death following exposure of one horn had no effect on the conceptuses of the contralateral shielded horn. Tempeature elevations in extrauterine tissues never exceeded those measured at the uterus.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on methods of interrupting pregnancy.

Proposed Course: This is a continuing contractual effort and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Morphochemical Analysis of Mucins in the Mammalian Oviduct
Contractor: University of New Mexico School of Medicine
Money Allocated: $17,763 (FY 70), $19,395 (FY 71), $17,523 (FY 72)

Objectives: The investigator plans to study the mucosubstances of each of the morphologically distinct regions of the oviduct from cycling and ovariectomized hormonally treated rabbits and from humans during the menstrual cycle by a battery of chemical staining techniques generally known to be specific for the reactive groups in the carbohydrate moieties of the epithelial secretions. The effects of specific chemical and enzymatic alterations of staining patterns will be analyzed to determine the reliability of observed staining alone. The ester sulfate, carboxylate and vicinal hydroxyl groups of the mucosubstances will be stressed in an attempt to correlate their presence or absence with various endocrine states. Various radioactive precursor sugars of epithelial mucins will be used to follow the incorporation of these radioisotopic labels into the oviductal mucins. This will be done under various hormonal states.

Major Findings: In the previous contract proposal the investigator had documented the pattern of mucins in the rabbit oviduct. Light microscope histo- and cytochemical techniques have shown that the mucins of the ampulla and isthmus had many highly acidic groups, whereas those of the infundibulum were weakly acidic. During the present contract year similar studies have been extended to the EM level and the regional distribution of various mucins has been confirmed. In post-coital rabbit oviducts there was observed a large increase in the amount of mucins. The regional differences observed in pre-coital tubes were maintained during post-coital intervals. In the ampulla and isthmus there is an accumulation of iron in the secretory granules during the first three post-coital days, with a subsequent decline. This suggests presence of acidic glycoproteins in the ampulla and isthmus.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the oviduct.

Proposed Course: This is a continuing contractual effort and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Steroid Hormone Binding in Reproductive Organs
Contractor: Yale University
Money Allocated: $39,141 (FY 70), $58,500 (FY 71), $67,692 (FY 72)

Objectives: The objective of this research project is to study various aspects of the interaction of contraceptive drugs and potential contraceptive agents with steroid binding molecules in organs concerned with reproduction. The investigator will continue studies directed towards *in vitro* comparisons of estrogen binding molecules from the rat uterus, vagina, anterior pituitary, and hypothalamus. The affinity of estradiol for binding to macromolecules from the supernatant fraction of these organs will be determined after *in vitro* incubation, and various drugs will be examined for their ability to compete with tritiated estradiol for macromolecular binding. To facilitate these studies, the investigator will attempt to develop a gentle and rapid purification procedure for estrogen binding macromolecules utilizing as the separating medium, estradiol reversibly linked to a standard chromatographic resin.

Major Findings: Estrogens bind *in vitro* to proteins in the supernatant fraction from the rat uterus, hypothalamus and anterior pituitary. It is thought that this corresponds to an initial interaction of estrogens with cytoplasmic binding proteins and is ultimately responsible for estrogen effects. The dissociation equilibrium constant for ^{3}H-estradiol is extremely low, 2×10^{-10}M. The two estrogens used in the oral contraceptives in the same doses have been studied for their binding. 17α-ethinyl estradiol binds about 400 times better than mestranol (3-methoxy-17α-ethinyl estradiol). This suggests that mestranol must be metabolized in the body to 17α-ethinyl estradiol for effective fertility control. The binding affinities of these two estrogens are similar in the uterus, anterior pituitary and hypothalamus.

In recent studies macromolecular binding of ^{3}H-estradiol has been shown using the supernatant from the liver. Although the binding levels are not as high as in the uterus they are much higher than in a non-specific organ such as the heart of the plasma. This interaction may be of importance in the initiation of some side effects since many of the complications of estrogen containing oral contraceptives may result from increases in plasma proteins (e.g. clotting factors, angiotensinogen) synthesized in the liver.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the steroid binding mechanism in reproductive and central nervous system tissues.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Immunologic Localization of Prostaglandins in the Reproductive Tract of Rabbit
Contractor: Yale University School of Medicine
Money Allocated: $39,850 (FY 72)

Objectives: The objective of this research is to study the localization of prostaglandins in the uterus, oviduct and on sperm during the capacitation process. Antibodies to prostaglandins will be coupled with peroxidase incubated with the respective tissues and subsequently reacted with diaminobenzidine. The last reaction produces electron dense material which can be subsequently studied with EM. The localization studies may provide an answer for the site of action or synthesis of prostaglandins at the cellular level.

Significance to Biomedical Research and Program of the Institute: The projected study on the localization of prostaglandins is directly related to the stated interests of the Contraceptive Development Branch in this group of substances.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Study of Progesterone-Binding Proteins as a Tool for the Detection of Progesterone Antagonists and Agonists.
Contractor: Institute of Medical Research of the Toledo Hospital
Money Allocated: $74,675 (FY 72)

Objectives: The purpose of this project is to prepare, isolate, purify and characterize a standard cell-free fraction containing receptor proteins from the uteri of rats, rabbits and guinea pigs incubated with ^{3}H-progesterone and from fresh untreated uteri in order to develop a rapid screening system for the detection of progesterone antagonists and agonists.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of progesterone binding substances in the uterus.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Prostaglandin: Its Role and Effect in Contraception
Contractor: University of California - Los Angeles
Money Allocated: $63,767 (FY 72) for two years

Objectives: The objective of this research is to study the role of prostaglandins on calcium uptake and release by the sarcoplasmic reticulum and mitrochondria from bovine and human uteri. The thesis is that the action of prostaglandins on uterine smooth muscle is due to the ability of these substances to regulate the intracellular stores of calcium. The primary aim of this research is to help to explain the mechanism of action of prostaglandins on the myometrium. Myometria from pregnant and non-pregnant cows will be fractionated to yield the sarcoplasmic reticulum and the mitochondrial fractions. Calcium transport in these fractions will be examined in the presence of various concentrations of prostaglandins. The proposed studies will be concerned with: (1) the effects of prostaglandins on calcium binding of bovine myometrial sarcoplasmic reticulum (SR) from non-pregnant animals at varying prostaglandin concentrations; (2) the role of progesterone in the sensitivity of uterine SR calcium transport to prostaglandins; (3) the interaction of prostaglandins with the SR membrane; (4) effects of prostaglandins on human myometrium as available; (5) the effects of prostaglandins on mitochondrial calcium binding in pregnant and non-pregnant animals

Significance to Biomedical Research and Program of the Institute: The projected study on the mechanism of action of prostaglandins is directly related to the stated interests of CDB in this group of substances.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Study of Progesterone-Binding Proteins as a Tool for the Detection of Progesterone Antagonists and Agonists.
Contractor: Institute of Medical Research of the Toledo Hospital
Money Allocated: $74,675 (FY 72)

Objectives: The purpose of this project is to prepare, isolate, purify and characterize a standard cell-free fraction containing receptor proteins from the uteri of rats, rabbits and guinea pigs incubated with ^{3}H-progesterone and from fresh untreated uteri in order to develop a rapid screening system for the detection of progesterone antagonists and agonists.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of progesterone binding substances in the uterus.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Prostaglandin: Its Role and Effect in Contraception
Contractor: University of California - Los Angeles
Money Allocated: $63,767 (FY 72) for two years

Objectives: The objective of this research is to study the role of prostaglandins on calcium uptake and release by the sarcoplasmic reticulum and mitrochondria from bovine and human uteri. The thesis is that the action of prostaglandins on uterine smooth muscle is due to the ability of these substances to regulate the intracellular stores of calcium. The primary aim of this research is to help to explain the mechanism of action of prostaglandins on the myometrium. Myometria from pregnant and non-pregnant cows will be fractionated to yield the sarcoplasmic reticulum and the mitochondrial fractions. Calcium transport in these fractions will be examined in the presence of various concentrations of prostaglandins. The proposed studies will be concerned with: (1) the effects of prostaglandins on calcium binding of bovine myometrial sarcoplasmic reticulum (SR) from non-pregnant animals at varying prostaglandin concentrations; (2) the role of progesterone in the sensitivity of uterine SR calcium transport to prostaglandins; (3) the interaction of prostaglandins with the SR membrane; (4) effects of prostaglandins on human myometrium as available; (5) the effects of prostaglandins on mitochondrial calcium binding in pregnant and non-pregnant animals

Significance to Biomedical Research and Program of the Institute: The projected study on the mechanism of action of prostaglandins is directly related to the stated interests of CDB in this group of substances.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Detection of Ovulation in Chronically Instrumented Animals
Contractor: University of Washington
Money Allocated: $45,600 (FY 72)

Objectives: The objective of this research is to devise a technique for sensing the arrival of a cumulus mass in the lower ampulla in chronically instrumented rabbits and primates. The arrival of a cumulus in the ampulla so closely follows the rupture of the ovarian follicle that detection of such an event would serve as detection of ovulation. The investigators propose to develop a cylindrical transducer in the shape of a cuff to be implanted around the oviduct at the ampullary-isthmic junction. Within this cuff on one side would be a piezoelectric crystal which would be stimulated to generate ultrasound and fill the oviductal lumen with ultrasonic radiation. Opposing this crystal would be a receiving crystal, and if a large mass such as the egg and cumulus passes through the ultrasonic radiation field, there would be a Doppler shift in the sound reflected back to the receiving crystal, resulting in a detectable change in the transmitted signal. Other changes that could be measured in the ultrasonic wave patterns produced by a moving object in the oviduct would be changes in the phase shift of reflected and backscattered sound, and plus echo phenomena. Various sources of false negative and positive indications of the moving cumulus would be excluded. Laparotomies or laparoscopies would be done at the time when various signals from the transducer suggest the presence of an egg and cumulus in the oviduct. The ultrasound can be amplified and modulated to produce audible signals so that the presence of a moving object such as an egg and cumulus in the oviduct would be signalled audibly to the investigators. The investigators propose to begin this work with rabbits and extend it to primates. The successful use of this technique would certainly lead to more accurate determination of the time of ovulation in experimental animals and facilitate the various experimental procedures in such animals. The first techniques would involve hard wire connections from the transducer, but ultimately they wish to explore development of a telemetering package for remote observation of unrestrained animals.

Significance to Biomedical Research and Program of the Institute: Accurate determination of the time of ovulation is of primary interest to the program of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of Techniques for Study of Oviduct Transport and Detection of Ovulation in Primates
Contractor: Case Western Reserve University
Money Allocated: $123,026 (FY 72) two years

Objectives: It is proposed to investigate the possibility of developing microelectronic sensors and radio-telemetry systems which can be totally implanted in the experimental animal to record several selected parameters believed to be related to ovulation. The proposed telemetry system will measure transovarian impedance, ovarian tubal impedance, ovarian size, ovarian temperature, and body core temperature. Other parameters, such as pressure, tubal motility and the electrical potential, could be telemetered with the same systems. The electrical impedance measurements are unique and have not been previously studied for the ovary, although the method has been successfully used in detecting changes in anatomical structure and histologic changes such as edema and hyperemia. It is believed that the transovarian electrical impedance changes will be easily seen through the ovarian cycle due to follicular growth and atresia. The follicular fluid in the growing follicle is isotonic with serum and therefore a good electrolyte so that the ovarian impedance will be low at the time of ovulation. During ovulation, when the follicle ruptures, there should be a sharp increase in electrical impedance. Following ovulation, when the ruptured follicle forms a corpus luteum, there should be a second increase in impedance. Correlative measurements of gonadotropin levels and basal body temperatures will be the indices of ovulation. In addition, measurement of ovarian temperature as compared to body core temperature will be carried out and it is expected that the differential between these two temperatures will increase during the follicular stage to the point of ovulation, whereupon the body temperature will rise, reducing the differential. For this project it is proposed to use telemetry systems previously developed in this laboratory and to modify them with special attention to the design and modification of transducers for this project.

Significance to Biomedical Research and Program of the Institute: Accurate determination of the time of ovulation is of primary interest to the program of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Assay of Progesterone Receptor Activity in the Guinea Pig Uterus
Contractor: Milton S. Hershey Medical Center
Money Allocated: $20,694 (FY 71), $23,544 (FY 72)

Objectives: The biological action of progesterone on the uterus may be initiated by the interaction of this steroid with a specific intracellular receptor protein. In the uterus of the castrate estrogen treated guinea pig specific cytoplasmic receptors for progestins have been identified. The purpose of this study will be to characterize these receptors in order to determine the best technique for measuring their binding capacities. A rapid assay technique will be developed for uterine cytosol protein binding activity. This assay will then be used to search for agents which both block progesterone binding to the cytosol receptor and which have relatively little progestational activity. From experience with other steroid receptor systems, it is anticipated that these agents will be antiprogestational and may therefore be useful as contraceptive agents.

Major Findings: The progesterone receptor in uterine cytosol is a protein that is present in a 3.5S form in the castrate and in a 7S form in the estrogen stimulated animal. The 7S receptor is dissociated to the 3.5S form by 0.1 normal KCl, heat, and gel filtration. The specificity of the cytosol binding protein for progesterone has been demonstrated by competition study with several steroids. Although cortisol is also bound to a 3.5S cytosol component, this steroid did not displace progesterone from either of its binding proteins.

Two separate assays were developed which were suitable for quantifying progesterone receptor activity: Cytosol is incubated with serial dilutions of radioactive progesterone. Bound and free steroid are separated either on Sephadex columns or by absorption with Florisil. Using the Florisil assays the mean association constant (Ka) the progestin binder is 2.3×10^{-8} and the number of sites in 5×10^{-9} moles per milligram of protein. When Sephadex filtration was employed the number of sites was unchanged, but Ka was 10 fold less. To explain the discrepancy in the estimation of Ka the protein fraction eluted from Sephadex columns was applied to a sucrose gradient and the progesterone labeled receptor migrated as a 2.5S macromolecule. These observations indicated that the procedure used for protein fractionation, in addition to temperature and ionic strength, may drastically influence the physical properties of the progestin receptor in guinea pig uterine cytosol. It is important to note, however, that even with change in size of the receptor the number of sites remains constant so that either assay can be used. Preliminary studies of a few progestational agents indicate that there is not a linear correlation with in vivo progestational activity and in vitro binding affinity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies on the binding characteristics of steroid receptor systems.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Role of Prostaglandins in Ovarian Physiology
Contractor: Yale University
Money Allocated: $25,470 (FY 71 Funds)

Objectives: The objective of this research is to study the effects of prostaglandins on ovarian physiology in the rhesus monkey. Specifically, this entails a study of 1) the effects of prostaglandins on steroidogenesis in the developing follicle and corpus luteum; 2) the effects of prostaglandins on development and morphology of the follicle and corpus luteum, and the effects on ovulation; 3) the effects of prostaglandin antibodies and antagonists on ovarian function.

Major Findings: Infusion of PGF_2 into monkeys during the luteal phase of the cycle had a biphasic effect on progesterone production. At low doses of PGF_2 there was a stimulation of progesterone output and at higher doses there was an inhibition. Infusion of PGF_2 during the follicular phase stimulated steroidogenesis as evidenced by increased estradiol levels in ovarian vein blood. Administration of estrogens during the luteal phase of the cycle increased the output of PGF_2 in the uterine vein blood.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the effects of prostaglandins on ovarian physiology.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Study of Neural Regulation of Ovarian Function
Contractor: Southwest Foundation for Research and Eduction
Money Allocated: $29,000 (FY 69), $31,146 (FY 70), $31,146 (FY 71), $40,271 (FY 72)

Objectives: The objective of the proposed study is to investigate the influence of the anti-inflammatory corticoid triamcinolone acetonide on the menstrual cycle of the baboon. The following end points will be explored: (1) dose-response relationship; (2) the effect on follicular development and on the endometrium; (3) the effect on luteinization when given immediately after ovulation, and (4) effect on the endogenous release of cortisol.

Major Findings: Triamcinolone acetonide when given during menstruation or early follicular phase consistantly blocked ovulation and shortened the menstrual cycle. Administration of the drug prior to expected ovulation produced equivocal results. Cortisol administered in the same way was without effect on the menstrual cycle. Aministration of triamcinolone during consecutive menstruations shortened the ensuing cycles.

Significance to Biomedical Research and Program of the Institute: The study of compounds that can block ovulation is directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: This is a continuing contractual effort leading toward the development of new contractives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Endocrine Regulation of the Corpus Luteum
Contractor: The University of Michigan
Ann Arbor, Michigan
Money Allocated: $26,706 (FY 69), $37,220 (FY 70), $53,618 (FY 71), $68,578 (FY 72)

Objectives: The primary objective of this research project is to elucidate the endocrine regulation of the ovine estrous cycle by measuring peripheral serum levels of FSH, LH, prolactin, estradiol, and progesterone. Radioimmunoassay methods are being developed for all these measurements. For the steroids, the methods are validated by comparison with double isotope derivative methods. Methods are also being developed to measure uptake of progesterone-C^{14} and LH-I^{125} by uterus and ovaries respectively. Preimplantation embryos will be studied to determine if they have the ability to synthesize LH in vitro, and to metabolize sex steroids in vitro.

Major Findings: Circulating levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PR) and progesterone (P) have been measured in samples collected at frequent intervals from cycling and pregnant ewes. There was no evidence that levels of LH or PR were increased during the mid-luteal phase of the cycle or early pregnancy, nor was there evidence of a decrease in these hormones during the late luteal phase of the cycle when regression of the corpus luteum occurs. FSH levels in serum were not increased during periods of active follicular growth. Progesterone levels were higher ($P<.001$) in ewes which were cycling normally or were pregnant than in ewes mated to fertile rams but which were not pregnant. Blood flow to the ovary which contains the corpus luteum increases 3-10 fold during the luteal phase of the cycle but remains constant throughout the cycle if no corpus luteum is present. Mixtures of antisera to LH and prolactin cause premature regression of the corpus luteum while either antiserum alone depresses progesterone levels but does not cause regression of the corpus luteum. Radioimmunoassays for FSH, prolactin and progesterone have been developed.

There was no evidence that significant arterio-venous differences exist across the ovary during various reproductive states. There was no evidence for the "utilization" of LH or prolactin during luteal phase nor FSH during the follicular phase.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to describe quantitatively the hormonal components of the estrous cycle in relevant animal models.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Anti-luteinizing Activity of Follicular Ovum
Contractor: University of Illinois
Money Allocated: $43,365 (FY 69), $45,635 (FY 70), $46,941 (FY 71), $39,834 (FY 72)

Objectives: Recent work from this laboratory has shown that removal of the egg and surrounding granulosa cells from a ripe ovarian follicle (rabbit) initiates luteinization and a short spurt in progesterone secretion. Hence, the egg seems to exert an anti-luteinizing activity. A series of well-planned studies to elaborate and determine the significance of this observation are being carried out under the terms of this contract.

Major Findings: Injection of porcine follicular fluid intravenously into rabbits for five days after mating has resulted in the following observations on day 16 of pregnancy:

1. A decrease in weight of corpora lutea (CL), a decrease in the number of living embryos, and a decrease in the percentage of living embryos. The partial correlation coefficient between the weight of CL and the percentage of surviving embryos at day 16 of gestation ($r=+0.676$) is significant ($P<0.01$). The analysis of variance of the effect of follicular fluids on embryo survival rate showed a significant ($P<0.01$) decrease in the percentage of living embryos (number of living embryos at day 16/number of implantation sites at day 8) due to follicular fluid effect as compared to rabbits injected with pig blood plasma (control). Porcine follicular fluid from the follicular phase of the cycle did not differ from that of the luteal phase with respect to their effect on the rate of embryo survival. Furthermore, rabbits injected with saline did not differ from those injected with pig blood plasma in percentage of living embryos.

2. A significant ($P<0.01$) decrease in progesterone concentration in the ovarian-vein-blood plasma in rabbits injected with follicular fluid was noted. Progesterone concentration and content in the luteal tissue of follicular fluid-treated rabbits were also less than in control rabbits treated with either saline or pig plasma. A similar decrease occurred in 20α-hydrox-4-pregnen-3-one in blood plasma from the ovarian vein and in the luteal tissue of rabbits treated with follicular fluid. The investigators have also analyzed the follicular fluid for LH, prostaglandins, estrogens and progesterone.

Significance to Biomedical Research and Program of the Institute: Reproductive function in the human appears to depend on corpus luteum luteinization and luteolysis which have thus been identified as particularly important topics to the purposes of the contraceptive development program.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Ultrastructural Studies of Estrogen Effects on Uterus, Oviduct, Ovary, Placenta, and Blood
Contractor: University of Minnesota
Money Allocated: $18,646 (FY 69), $20,865 (FY 70), $23,357 (FY 71)

Objectives: This is a wide-ranging study of the reproductive tract by ultrastructural and radioautographic methods. The fine structure of the corpus luteum of the white-tailed deer and raccoon is being studied, and several species of animals (deer, raccoon, and green monkey) are being injected with estrogen during the anestrous and estrous periods, and samples taken at all levels of the female reproductive tract. These studies will relate ultrastructural cytology with biochemical functions, with particular emphasis on the evolutionary significance of hormonal changes.

Major Findings: Analysis of blood plasma proteins of the green monkey indicate that the observed pattern of changes in this species in response to contraceptive agents is very similar to the observations in the human.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Corpus Luteum Regulatory Mechanisms
Contractor: University of Western Ontario
London, Ontario, Canada
Money Allocated: $36,508 (FY 69), $45,208 (FY 70),
$47,232 (FY 71)

Objectives: This is a study of the luteinization process of the granulosa cell at the time of ovulation, as well as of the process of luteolysis. The investigator previously has shown that autotransplantation of follicles to beneath the kidney capsule enables the process of luteinization to be studied independently of changes which may occur in other ovarian components. Using this approach he is investigating the role of LH in luteinization. If LH produces luteinization, possible intermediates in the action of LH such as cyclic AMP, RNA, and protein fractions from LH-treated follicles can then be substituted. To test the hypothesis that luteinization of granulosa cells may be secondary to increased permeability of the basement membrane, follicles are pre-incubated in diluted solutions of hyaluronidase. Finally, the influence of steroids such as progesterone, dihydroprogesterone, estrogens, etc., is being evaluated by the addition of the steroids to the follicles during a pre-incubation.

A study of luteolytic factors is also being conducted in the rat, guinea pig and rabbit in a search for a species which models the human in its biosynthesis of steroids and its steroid phospholipid and intracellular localizations. LH, LTH, estrogen, progestins and hypophysectomy are being examined for their effects on corpus luteum function and regression.

The human corpus luteum will be studied by incubation of corpora lutea removed surgically in normal cycles and after the administration of estrogens and progestins. Plasma LH and progesterone will also be determined. The luteotrophic effects of estrogen will be assessed as well as estrogen inhibitors.

Major Findings: Further studies in which ovarian follicles of rats have been incubated with dibutyryl cyclic AMP have confirmed the ability of this nucleotide to mimic the action of LH in initiating luteinization of follicles subsequently transplanted to hypophysectomized rats.

Blockade of cholesterol side-chain cleavage with the drug, amino-glutethemide phosphate, did not prevent luteinization induced by LH. Addition of Actinomycin D to the incubation medium in the presence of LH similarly failed to prevent LH-induced luteinization.

Prolactin, at dosages which inhibit ovulation and luteinization, inhibits the conversion of progesterone in non-lutein tissue (follicle and interstitial cells) to two products which have now been definitively identified as 20α-hydroxy-pregn-4-en-3-one and 5α-pregnan-3α,20α-diol.

In order to investigate the possible binding of progesterone metabolites by endometrial proteins, methods for the synthesis of 5α-pregnan-3, 20-dione-7α-^{3}H and 3α-OH-5α-pregnan-20-one-^{3}H have been developed. Preliminary studies have revealed that a cytosol fraction from pregnant rat endometrium has 2 - 3 X the binding affinity for this metabolite as for progesterone. Studies of the specificity of this binding have been initiated.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: Renewal not requested.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Hypothalamo-Hypophysial Factors in Reproduction
Contractor: Medical College of Ohio at Toledo
Money Allocated: $82,911 (FY 69), $70,555 (FY 70), $56,700 (FY 71)
$36,000 (FY 72)

Objectives: The principal objective of this research is to develop a reliable and sensitive displacement assay for oxytocin for the purpose of eventually measuring blood levels of this peptide during the menstrual cycle and following coitus. Using this technique, the investigator is assaying oxytocin levels in animals during the estrous cycle and after mating and will eventually apply the method to determining blood levels of this hormone in women. In addition, the structural elements of the oxytocin-binding peptide are being examined and analogues of oxytocin are being synthesized and compared with oxytocin with regards to their ability to bind to the binding peptide.

Major Findings: Uptake of radioactive oxytocin by mammary glands (M.G.) has been investigated. Strips of M.G. take up oxytocin in a linear fashion. The affinity of the binding fraction to oxytocin was low. Several oxytocin analogs compete with oxytocin for the binding sites.

Work is also reported on binding of oxytocin to the uterus. This work is very preliminary but it does indicate displacement of radioactivity by cold oxytocin but not by an inactive analog. Preliminary studies have begun on the subcellular localization of radioactive oxytocin. Experiments were begun on the antifertility effect of oxytocin in pregnant rats. Administration of oxytocin to pregnant rats reduced litter size and had a paradoxical effect on sex ratio.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch to support research directed towards the elucidation of the hypothalamo-hypophysial control of reproductive function.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Actions of Estrogen and Progesterone upon Uterine Function in Pregnancy
Contractor: Washington University
St. Louis, Missouri
Money Allocated: $46,329 (FY 69), $48,718 (FY 70),
$48,718 (FY 71), $44,030 (FY 72)

Objectives: This investigation involves three projects: (1) Removal of corpora lutea in early human pregnancy and observations of plasma progesterone levels, uterine activity and abortion. Progesterone determinations are also made on the enucleated corpora lutea and the placenta. For patients coming to surgery after the first half of the first trimester, pregnancy is terminated by the intra-amniotic injection of hypertonic saline and the effects of this treatment on plasma progesterone and uterine activity are studied. (2) The role of placental estrogen in luteal maintenance is being studied in rabbits and rats with emphasis on questions of timing, dose, duration in treatment and species differences. (3) Electrophysiological studies being conducted on the rabbit uterus and oviduct under a variety of hormonal conditions include: tension measurements in electrically stimulated uterine and oviduct strips; measurements of differential shortening of stimulated muscle strips; and direct measurements of action potentials to establish the extent and velocity of conduction in uterine and oviduct muscles. These studies will be extended to observations of the human Fallopian tube.

Major Findings: The effects of luteectomy in women averaging 50.8+1.0 days of gestation (Nagele) were studied with respect to induced abortion, progesterone levels and the development of intrauterine pressure. Only 3 of 11 women undergoing luteal excision had complete abortion, the others varying from incomplete to incipient. This variability appeared related to the extent of placental progesterone production at the time of luteectomy.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the importance of the corpus luteum in early human pregnancy and the timing of the luteal-placental shift.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: New Methods for Fertility Regulation
Contractor: The Upjohn Company
Money Allocated: $127,684 (FY 69) for two years, $73,425 (FY 71)
$69,887 (FY 72)

Objectives: This is a study of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in which the conditions under which $PGF_{2\alpha}$ causes luteolysis and induces the termination of pregnancy will be determined, and the mechanisms by which $PGF_{2\alpha}$ achieves its luteolytic effect will be identified. Pregnancy termination is being studied in rabbits, guinea pigs, and rhesus monkeys by ovarian histology and ovarian and peripheral plasma progesterone levels. The period of pregnancy subject to interruption by $PGF_{2\alpha}$-induced luteolysis is being established.

The second aspect of the proposal is an attempt to study the processes involved in the luteolytic effects of $PGF_{2\alpha}$. This includes studies of effects on gonadotrophin activity, competitive inhibition of gonadotrophins, direct effects on progesterone synthesis in the ovary by measurement of steroid levels in ovarian venous blood, and of the effects of prostaglandins on steroidogenesis by rat ovaries and primate corpora lutea in vitro. This also includes studies of cholesterol incorporation into progesterone, levels of adenyl cyclase, inhibition of effects of puromycin and cycloheximide, and potentiation of effects by phosphodiesterase inhibitors.

Major Findings:
(1) Following the administration of $PGF_{2\alpha}$ to rhesus monkeys ovarian and uterine blood flows as determined by hydrogen desaturation were depressed dramatically. The effect lasted for 1.5 to 2.5 hours after injection. Administration of this dose of $PGF_{2\alpha}$ increased arterial blood pressure, peripheral resistance, pulmonary arterial pressure and venous pressure slightly, and decreased heart rate slightly. These parameters tended to return toward control levels over a thirty-minute period, post treatment.

(2) The in vitro binding of ^{3}H-$F_{2\alpha}$ to segments of uterine tissues was demonstrated. In the pseudopregnant hamster degree of binding was highest on days 3 and 4. The percentage of inhibition by cold $F_{2\alpha}$ was higher and more consistent on days 5 and 6 and 7 of pseudopregnancy.

(3) Cytosol binding of $PGF_{2\alpha}$ to uterine proteins could not be demonstrated.

(4) Using the superfusion technique the investigators have demonstrated de novo synthesis of $PGF_{2\alpha}$ by the ovary and the uterus. In the uterus synthesis were greater in estrous than pseudopregnant rabbits.

(5) A dramatic diurnal pattern in the serum concentration of $PGF_{2\alpha}$ was observed in female monkeys during the luteal phase but not in the follicular of the menstrual cycle; serum $PGF_{2\alpha}$ was highest at 0600 and 1200 hours and lowest at 1800 and 2400 hours. The diurnal pattern in serum cortisol concen-

tration did not coincide with the pattern for $PGF_2\alpha$ and was unaffected by stage of the menstrual cycle. Male rhesus monkeys exhibited a diurnal pattern in serum $PGF_2\alpha$ concentration similar to that observed in the luteal phase, female monkey. The data indicated that blood levels of prostaglandin may be regulated by the gonad.

Significance to Biomedical Research and Program of the Institute: The relevance of this study to the contract program is direct, particularly insofar as some of the work is being done in primates.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Isolation of a Bovine Uterine Luteolytic Factor
Contractor: Cornell University
Money Allocated: $107,232 (FY 69), for three years,
$43,687 (FY 72)

Objectives: The principal investigator has prepared a bovine endometrial extract with the ability to regress corpora lutea of pseudopregnant, hysterectomized hamsters. The active material precipitates with ammonium sulfate and apparently is a large molecular weight protein; the possibility is being investigated that it may be prostaglanding $F_2\alpha$ adsorbed to a protein. These studies involve further fractionation and purification of the active material using preparative disc electrophoresis, and study of its effects on corpus luteum progesterone synthesis stimulated by LH, the effects of various modes of administration, and effects on ovarian blood flow and progesterone concentration.

Major Findings: A luteolytic protein has been obtained from the cytoplasmic fraction of bovine endometrium which has been further purified by ammonium sulfate precipitation, hydroxylapatite chromatography and preparative electrophoresis. Incubation with pronase decreased the luteolytic activity of this protein, but heating did not. Purification of this protein has not removed steroid (progesterone) binding properties. Luteolytic activity can be removed by lipid extracts, but the nature of this lipid extract is not yet clear. Attempts to identify this lipid as $PGF_2\alpha$ have not been satisfactory, and it has also not been possible to isolate PGF at levels sufficient to detect in the gerbil colon assay. Similar extracts from hamster uteri were active in the gerbil colon assay and luteolytic when administered to hamsters. $PGF_2\alpha$ had two distinct effects in the hamster, being luteolytic at high concentration and luteotrophic at low concentration. Evaluation of endometrial fractions, active in the hamster, are continuing in the cow. Until recently, the luteolytic effect in the cow has been equivocal.

Significance to Biomedical Research and Program of the Institute: Corpus luteum regression, or "luteolysis" has been identified as particularly important to contraceptive development; therefore, this study is directly relevant to the purposes of the program.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Identification of the Luteotrophic Complex in the Ewe
Contractor: University of Wyoming
Money Allocated: $39,820 (FY 69-70), $21,252 (FY 71)

Objectives: The investigator is continuing studies on luteal maintenance in the hypophysectomized ewe. He is attempting to identify the nature of the unknown material in crude LH preparations which, on constant IV infusion, maintains corpora lutea in a functional state in hypophysectomized ewes, while purified LH preparations are unable to do so. Various combinations of ovine LH, FSH, prolactin, TSH, ACTH, and GH are used. Measurements made include twice daily peripheral plasma progesterone levels, and size and progesterone content of corpora lutea removed at the end of the eight-day experimental period.

Major Findings: Pregnant ewes hypophysectomized on day 22 or 23 of gestation do not maintain their pregnancies when given only saline infusion (5 out of 5 ewes). Hypophysectomized pregnant ewes infused with 2.5 mg/day of pure LH also failed to maintain the pregnancy for the period of infusion. Combination of LH with prolactin (2.5 mg/day) maintained pregnancy in 3 out of 4 ewes.

Significance of Biomedical Research and Program of the Institute: Although, because of the apparent differences which exist between species, it is not clear that knowledge of the process in sheep will by itself be of assistance in determining the factors maintaining luteal function in the human, it is clear that a more rational approach to the induction of luteolysis can be made if the nature of luteotrophic factors is known.

Proposed Course: Renewal not requested.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Control of Ovulation in the Monkey
Contractor: Oregon Primate Center
Money Allocated: $44,000 (FY 69) for two years, $47,543 (FY 71)
$56,751 (FY 72)

Objectives: These studies are directed towards establishing the feedback site of progestins as inhibitors of ovulation in the rhesus monkey using experimental approaches previously used in the rabbit. These approaches are feasible in the rhesus monkey but much base-line information is being obtained, preliminary to critical studies of feedback inhibition of ovulation.

Major Findings: The investigator has characterized the pattern of circulating LH and FSH throughout the menstrual cycle of the rhesus monkey. Both progesterone and an antiestrogen (U11 100A) blocked the mid-cycle surge of LH and ovulation when injected during the second half of the follicular phase.

Stalk median eminence extracts (SMEE) but not cerebral cortical extracts of sheep caused a marked increase in serum LH following I.V. injection. Purified LH-RH was not effective when administered systemically at stages of the cycle and at dosages known to elicit an LH rise in humans. LH-RH did cause a significant elevation of serum LH following direct pituitary infusion.

Significance to Biomedical Research and Program of the Institute: Studies of the mechanism of ovulation are clearly relevant to the purposes of the contraceptive development program.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Contraception through Disordering of Luteal and Uterine Events
Contractor: Harvard Medical School
Money Allocated: $158,441 (FY 69) for three years

Objectives: The major aim of this program is to determine the hormonal factors regulating the mammalian corpus luteum - its growth and regression. Experiments being carried out under this contract deal with:

1. the significance of cholesterol esterases in normal, unsuperovulated ovaries during different functional states;
2. development of testing methods for measuring oxygen consumption in intact CL;
3. effects of LH on steroidogenesis in mouse Leydig cell tumors;
4. pituitary luteotropic and luteolytic activity;
5. direct action of progesterone on the ovary;
6. uterine luteolytic activity in guinea pigs;
7. influence of IUD's on CL function.

Eventually, findings in rats and guinea pigs will be tested in primates.

Major Findings: The investigators have shown that the steroidogenic activity of the rat ovary is closely related to the turnover of cholesterol esters and the activity of the enzyme cholesterol ester synthetase. Esterified cholesterol must be hydrolyzed to free cholesterol before being available for steroid synthesis. LH has an action to increase the conversion of esterified cholesterol to free cholesterol within ten minutes of incubation.

Significance to Biomedical Research and Program of the Institute: This is a comprehensive program the objectives of which are directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: Renewal not requested.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Relationships of Endometrial Prostaglandins and Luteal Function
Contractor: West Virginia University
Money Allocated: $35,337 (FY 69) for two years, $32,460 (FY 71), $33,899 (FY 72)

Objectives: The investigators are extracting the endometrium of sheep at various phases of the cycle to determine prostaglandins both qualitatively and quantitatively. The effects of progesterone on the corpus luteum and on prostaglandin levels and the effects of prostaglandin $F_{2\alpha}$ on corpus luteum function in hysterectomized animals are being studied. Corpus luteum function is being evaluated by progesterone levels and length of estrous cycle.

Major Findings: Experiments completed have shown that endometrial levels of prostaglandin (PG) $F_{2\alpha}$ are increased (1) at day 14 of the estrous cycle, (2) at day 5 after insertion of bilateral IUD's on day 2 and (3) slightly but significantly on days 5 and 9 after progesterone treatment on days 0 and 1. In ovariectomized ewes, daily injection of progesterone decreased levels of $PGF_{2\alpha}$ ($P<0.05$). Unexpectedly, pregnancy caused a dramatic, significant increase in endometrial and uterine venous $PGF_{2\alpha}$ as measured on day 13 post-estrus. Five pregnant and 5 non-pregnant ewes have been sampled at days 11, 12, 13, 14, 15 and 16 postestrus as well as 5 pregnant ewes at day 18 and endometrial samples analyzed to date indicate the same trend at days 12, 14, 15 and 16 as at day 13.

Samples have been collected for the comparison of the caruncular and inter-caruncular areas of the sheep endometrium.

A preliminary study of monkey endometria indicates presence of prostaglandins $F_{2\alpha}$ and E_2.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to elucidate the factors responsible for the control of corpus luteum function.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Effect of Progestational and Estrogenic Hormones on the Uterus
Contractor: Albert Einstein College of Medicine
Money Allocated: $124,274 (FY 69) for two years, $62,137 (FY 71)

Objectives: The main objective of this research project is to determine the role of progesterone in the biochemical and morphologic changes in the rabbit endometrium and myometrium which take place following ovulation. Principal parameters being studied include cytochrome content of mitochondrial and microsomal fractions, total DNA, RNA and protein, uptake of labeled precursors, O_2 uptake, and endometrial enzymes. Appropriate biochemical and ultrastructural techniques are being applied to these measurements.

Major Findings: Studies on the effects of progesterone and 17β-estradiol demonstrate a striking difference. Estrogen appears to stimulate the entire uterus in a similar manner not differentiating between endometrial and myometrial cells as far as the total nucleic acid and protein contents of these tissues are concerned. In contrast, progesterone stimulates DNA synthesis in the endometrium but not to any appreciable extent in the myometrium.

With estrogen treatment, the synthesis of an "induced protein" reported by other investigators appears to take place in the rabbit endometrium.

Cells from hormone stimulated endometrium have been separated into three types, examined in the electron microscope, and maintained in culture.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to determine the mechanism of action of ovarian hormones on uterine metabolism and morphology.

Proposed Course: Renewal not requested.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Effect of Autonomic Drugs on FSH and LH Releasing Factors
Contractor: The Ohio State University
Money Allocated: $41,887 (FY 69) for three years

Objectives: The objective of this research project is to study the release of LH by pituitary halves incubated with median eminence slices and hypothalamic minces, and to evaluate the inhibitory effects of various drugs. In a second series of experiments, drugs will be injected into the ventricular system of the rat brain and LH release evaluated.

Major Findings: The suppression of FSH and LH secretion by estrogen and progesterone differs in reserpine-pretreated from non-reserpine-pretreated rats. This suggests that biogenic amines modulate steroid effects on the brain. The systemic administration of L-Dopa produced FSH release in animals pretreated with progesterone and LH release in animals pretreated with estrogen, suggesting again interaction of steroid and biogenic amines in this response. When biogenic amines are injected intraventricularly, dopamine produced FSH release under most conditions and norepinephrine inhibited FSH release. When injected intraventricularly, atropine produced marked inhibition of FSH release, while hexamethonium stimulated FSH secretion. This suggests that there are both stimulatory (muscarinic) and inhibitory (nicotinic) receptors involved in FSH secretion. Tremorine, a muscarinic agonist, inhibited LH secretion when administered systemically.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: Termination.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Physiological Aspects of the Attachment of Carbohydrate to Luteinizing Hormone
Contractor: University of Texas
Houston, Texas
Money Allocated: $119,340 (FY 69) for three years, $58,464 (FY 72)

Objectives: The investigator is collecting sheep pituitary glands and classifying them according to sex or age of the animal in order to determine whether these parameters affect the carbohydrate subunit and its attachment to the luteinizing hormone molecule. Various theoretical considerations have led to the postulate that LRF activity may be defined as the process of activating a particular type of carbohydrate attachment to the protein core of LH in the pituitary. The data being collected in this study will go far toward testing this postulate. This program is of interest in connection with studies of ovulation control and corpus luteum formation, in addition to its primary importance in helping to clarify the chemistry of luteinizing hormone.

Major Findings: The investigators have developed methods for batch processing large quantities of sheep pituitary tissue, recovering LH with high biologic specific activity, and dissociating this latter substance into "homogeneous" preparations of subunits. Using these preparations, the primary structure of ovine LH subunits has been determined.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to elucidate the mechanism of action of the hypothalamic-releasing factors and the pituitary hormones.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Progesterone Production and Metabolism during the Female Reproductive Cycle
Contractor: Case Western Reserve University
Cleveland, Ohio
Money Allocated: $30,430 (FY 70), $34,711 (FY 71)

Objectives: The first objective of this project is to study the plasma metabolic clearance rate and plasma production rate of progesterone and 20α-hydroxypregn-4-en-3-one in human subjects at the mid-follicular phase, the mid-cycle period, and the mid-luteal phase of the menstrual cycle. These values will be correlated with plasma levels of luteinizing hormone as measured in the same patients and the percent conversion of progesterone in vivo to 20α-hydroxypregn-4-en-3-one will be calculated. Competitive protein binding methods will be employed to measure plasma concentrations of both steroids.

A second aspect of this research involves evaluation of the influence of plasma protein binding on the metabolism and distribution of progesterone. To study the effect of steroid binding to transcortin on the steroid distribution and metabolism, monkeys will be infused with tritiated progesterone, either in the absence or presence of purified human transcortin. In addition to measuring metabolic clearance rate in these animals, hepatic extraction of free and bound progesterone will be measured in monkeys with catheterized hepatic veins. It is also proposed to study the influence of transcortin on the uptake of tritiated progesterone from the plasma by a variety of tissues, in rats. Metabolic products of progesterone found in these tissues will also be measured.

Major Findings: The plasma metabolic clearance rate (MCR) of progesterone, determined by the continuous infusion method, was 2580 ± 176 (SE) L/day in 15 women at various times of the menstrual cycle. There was no correlation between the day of the cycle and the MCR value. The MCR's determined three times in the same cycle in three women at the midfollicular, midcycle and midluteal phases were 2270, 2620 and 2570 L/day; 2460, 2520 and 1890 L/day; and 2230, 3480 and 3300 L/day. The MCR's determined every other day at midcycle in three women were 1850, 1850 and 1800 L/day; 2070, 3300 and 2010 L/day; and 1750, 2010 and 1930 L/day. The combined plasma MCR of progesterone for the 21 women was 2510 ± 134 (SE) L/day. The conversion ratio of progesterone to 20α-hydroxypregn-4-en-3-one was 10.4 ± 0.67 (SE) per cent with no change during the cycle.

The blood MCR OF 20α-hydroxypregn-4-en-3-one in three women was 3140, 4140 and 2710 L/day. In the third subject the MCR was determined on days 9, 18 and 23 of a 37 day cycle to be 2860, 2560 and 2720 L/day, respectively.

New methodology for 20-α-hydroxy progesterone measurement has been developed.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of ovarian steroid metabolism and production during the menstrual cycle in primates including humans.

Proposed Course: Termination.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Control of Ovulation and Corpus Luteum Function
Contractor: Medical College of Georgia
Money Allocated: $43,760 (FY 70), $25,891 (FY 71), $33,492 (FY 72)

Objectives: The investigators propose studies to gain insight into the action of estrogens at the hypothalamic and pituitary level. More specific studies will deal with steroid-protein interaction in the rat hypothalamus and pituitary and in the human pituitary. This work will involve the use of both radioactive estradiol and testosterone and the possible identification of the testosterone metabolite which binds to the cytosol fraction. This study will include the use of hormone analogs that bind covalently to the 8S-receptor.

Major Findings: Cytosol fractions from the pituitaries of male and female rats were shown to have cytoplasmic receptors of high affinity for estradiol. Sephadex G-200 gel filtration, sucrose density gradient analysis and equilibrium dialysis techniques indicated that this receptor was primarily the 8S-receptor and the binding capacity in the two sexes were identical. The binding of testosterone by male and female pituitary cytosol fractions was low and essentially non-specific. Dihydrotestosterone had an immediate level of binding. Preliminary work with hypothalamic fractions showed a similar pattern. These results, when viewed with the effects of various steroids on serum gonadotropins in castrated male rats, suggest that gonadotropin suppressing effect of testosterone may be mediated through its conversion to estrogen and/or dihydrotestosterone. The cytoplasmic receptors for estradiol and low or absent binding for testosterone was not unique for the rat. Similar observations were made in a human pituitary tissue sample which appeared normal histologically.

The investigators were not successful in their studies of hormone effects on protein synthesis in the pituitary in vivo. In vitro incubation of pituitary tissue with estradiol and testosterone resulted in decreased incorporation of ^{3}H methionine into protein.

Studies with 4-mercuri-17β-estradiol have indicated a new interesting finding. This compound is a good estrogen that binds covalently to the 8S-cytosol receptor in the uterus and does not subsequently become associated with nuclear receptors. These findings implicate a direct role of 8S-cytosol binding in manifestation of biological activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the action of the pituitary gonadotropins in the reduction of ovulation and the control of corpus luteum formation, function, and regression.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Development of Inhibitors to Estrogen Biosynthesis
Contractor: Worcester Foundation for Experimental Biology, Inc.
Money Allocated: $43,634 (FY 70),.$43,634 (FY 71), $59,603 (FY 72)

Objectives: In previous work the investigator has shown that the conversion of androst-4-ene-3,17-dione and testosterone to estrogens involves reaction sites at carbon 1, 2, and 19, and that these sites are not utilized in other important steroid metabolic processes. Based on these findings, this research project is devoted to developing specific inhibitors to the aromatase enzyme system which catalyzes the production of estrogens. The investigator seeks a specific, effective inhibitor which may result in lowered estrogen levels in vivo. The enzyme system will be prepared from human placentas utilizing a standard preparation with appropriate controls.

Major Findings: A systematic evaluation of the ability of steroids containing various functional groups to inhibit estrogen biosynthesis is being carried out. Androstenedione-4-^{14}C is incubated with human placental microsomes in the presence and absence of a potential inhibitor and the exact amount of estrogen produced in each case is determined with the aid of tritiated estrogens as recovery markers.

In general, structures that were good inhibitors of estrogen biosynthesis were structurally related to substrate. The better ones were androsta-1,4,6-triene-3,17-dione, 17β-acetoxyandrosta-4,6-dien-3-one, androst-4-ene-3,6,17-trione and testosterone formate. All inhibited estrogen biosynthesis from 75%-90% at less than 1 mM concentration.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the biosynthetic pathways for ovarian steroids with special attention being paid to possible points of inhibition for the purpose of fertility control.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Role of Uterine Luteolytic Substances in Reproduction: An Evaluation of the Specificity of Porcine Granulosa Cell Monolayers as an Assay System
Contractor: Duke University Medical Center
Money Allocated: $17,070 (FY 70), $17,070 (FY 71)

Objectives: This investigator's previous finding that uterine flushings of pigs obtained during luteal regression rapidly destroy granulosa cells cultured in vitro, whereas flushings from the earlier portions of the estrous cycle do not, has been criticized because the specificity of this lytic action has not been established; on the contrary, there is evidence that it may be quite non-specific. The investigator now proposed to undertake a thorough study of the specificity of his cell culture assay system. Extracts from various tissues as well as a number of enzymes will be used. Conversely, the activity of uterine extracts in cell cultures of various non-ovarian tissues will also be examined. Lytic activity will be evaluated by time-lapse cinematography.

Major Findings: Using Sephadex G-200 columns the material from the uterine flushings was characterized as a high molecular weight substance. Studies of tissue extracts from skeletal muscle, heart, liver, spleen, pancreas, lung, ovarian interstitium and kidney did not produce a similar degree of lysis as did the luteal phase uterine flushings. A number of enzymes, progesterone, estradiol 17β and $PGF_{2\alpha}$ could not duplicate data obtained with flushings. Extracts of endometrium were not lytic when the amount of extract protein was held to 10 mg/culture. At higher concentrations of protein, endometrial extracts were highly lytic. Flushings on the other hand were lytic when added to culture at the equivalent of 1.5 mg/culture. The amount of lysis induced by endometrial flushings was quantitatively diminished when fetal calf serum was substituted for pig serum in the culture medium.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies devoted to elucidating the factors responsible for luteal regression.

Proposed Course: Terminated.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of Radioimmunoassays for Rhesus Monkey Gonadotropins
Contractor: Endocrine Laboratories of Madison, Inc.
Money Allocated: $78,553 (FY 70), $78,553 (FY 71)

Objectives: This proposal is based on the recognition that rhesus monkey gonadotropic hormones do not cross-react immunologically with their human counterparts or at least not sufficiently to permit their detection in body fluids by radioimmunoassay. In this project, the investigators will collect urine from gonadectomized rhesus monkeys for the isolation of monkey LH and FSH. The urinary gonadotropins will be utilized because of the limited availability of monkey pituitary glands. The gonadotropins thus obtained will be used as antigens to obtain antisera for development of radioimmunoassays. Purification of the hormones will be by procedures previously developed, and by adaptations of methods developed in other laboratories and reported in the literature.

Major Findings: The work has been concerned primarily with (1) stock-piling extract of urines from ovariectomized monkeys; (2) exploration of assays of a pooled rhesus monkey urine extracts in comparison with a number of other gonadotropin assays for LH, FSH and total gonadotropins, (3) variation in the extraction procedure to see what are the optimal conditions for the recovery of FSH and LH.

Initial assays of individual monkey urines have allowed the separation of the ovariectomized colony into 4 groups: high LH, medium LH, high FSH and medium FSH. The urines were collected under refrigeration, acidified and then stored at -20°C. The two primary procedures that have resulted in large quantities of crude gonadotropins have been the kaolin-acetone extraction procedure and the ultrafiltration procedure. A total of 1,468 liters of urine have been extracted with anticipated activity equivalent to 275 to 300 mg of NIH-FSH and total LH activity equivalent to approximately 40 to 50 mg of NIH-LH. Comparing different FSH assays the investigators have concluded that the Steelman-Pohley assay was most advantageous. In testing the potency of extracts, it appears advisable to acidify during collection if the urine is to be used for FSH extraction.

For LH assays the investigators used the OAAD test ad described by Bogdanove and Gay. Using this assay it was determined that kaolin-acetone extraction and ultrafiltration recovered approximately equivalent amounts of LH activity. The direct acetone precipitation, and direct acetone precipitation plus kaolin-acetone extraction, appears to yield approximately three to ten times that obtained by kaolin-acetone extraction.

The urinary LH is immunoreactive and the investigators are attempting to purify the material for use in a RIA.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support the development of new methodologies for the assay of steroid and protein hormones in the blood of primates including man.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Pilot Study for the Large Scale Preparation of Purified Human Chorionic Gonadotropin
Contractor: Columbia University
Money Allocated: $47,300 (FY 70), $85,086 (FY 71), $194,280 (FY 72) for two years

Objectives: The objective of this investigation is to undertake a feasibility study to determine the optimal conditions for preparation of large batches of highly purified human chorionic gonadotropin (HCG). Secondary goals of this program are: (1) to obtain data bearing on the chemical and immunological characterization of HCG fractions which appear to be homogeneous in polypeptide content, but possess differing biological activities, and (2) to obtain data on the stability of HCG using both immunological and biological activities as guides.

Major Findings: Methods have been developed to prepare highly purified human chorionic gonadotropin and also purified preparations of the hormone's alpha and beta subunits. A radioimmunoassay developed for the HCG-beta subunit has proved to be highly specific for detection of HCG - even in the presence of luteinizing hormone. Potential applications include the capability of detecting the presence of pregnancy before the first missed menstrual period and improvements in the detection and the chemotherapy of choriocarcinoma. A quantity of this purified HCG - beta subunit has been prepared for distribution to investigators in the field of population research for use in this radioimmunoassay application. Quantities of purified HCG and of the two subunits are being distributed by the National Pituitary Agency.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support the development of procedures for human hormone isolation and purification.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: A Simultaneous Theoretical and Empirical Approach to the Study of the Rat Estrous Cycle
Contractor: University of Illinois
Money Allocated: $41,500 (FY 70), $45,226 (FY 71), $71,713 (FY 72)

Objectives: The investigator plans a systems analysis approach to the understanding of the mechanisms regulating reproductive cyclicity and acyclicity in the rat. The total approach includes the following stages to be carried out simultaneously: identify from existent experimental data the essential variables and connecting linkages among system components; make a model of the system; simulate the system by computer; perform "experiments" using the model and simulating by computer to compare the model to similar experiments in the real system; use the computer to simulate the results of new experiments; perform these experiments and modify the model if necessary, etc.

Because data on few of the variables needed for constructing an adequate simulation are available from the literature, the investigator will measure the following parameters and variables using radioimmunoassay and competitive protein binding methods for steroids and gonadotrophic hormones: (1) estrogen, progesterone, LH, FSH, and prolactin in serum throughout the four-day cycle; (2) distribution volumes and loss rate constants of the above hormones in cyclic rats and following ovariectomy and/or hypophysectomy; (3) setpoints for LH, FSH and prolactin in prepubertal and adult rats; (4) the negative feedback constant for the suppression of LH and FSH secretion by estrogen; (5) ovarian transfer functions, determining the effects of gonadotropins on estrogen and progesterone secretion and on morphology; (6) estrogen, LH, and FSH levels during the onset, steady state and termination of persistent estrus due to continuous light.

Major Findings: The computer simulation of the rat estrous cycle has been rewritten in EQUAL, for the APL/360 time-sharing system. Parameter values have been updated in terms of empirical data. Rates of LH production have been empirically measured in intact and ovariectomized rats of different ages and intervals after ovariectomy. Age and operative were more important in determining LH production rate than number of days after ovariectomy. Serum LH and progesterone have been measured during the afternoon of proestrus in four-day and five-day rats; the LH appears more abruptly and the peak lasts for a shorter time in four-day rats. Preliminary measurements of serum estradiol and progesterone have been made in the morning of the two days proceeding proestrus in both kinds of rats.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the mechanisms regulating reproductive cyclicity and acyclicity.

Proposed Course: This is a continuing contractual effort and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Biochemical Life Cycle of the Rabbit Corpus Luteum and its Modification by Humoral Factors
Contractor: Medical University of South Carolina
Money Allocated: $21,493 (FY 70), $5,350 (FY 71)

Objectives: The life cycle of the rabbit corpus luteum will be studied with respect to several biochemical parameters, all of which are related to the ultimate function of this organ, elaboration of progestagens. The initial parameter to be investigated is adenyl cyclase activity which will be studied in the rabbit corpora lutea at varying times after ovulation and luteinization have been induced by the administration of luteinizing hormone (LH). Adenyl cyclase will be determined in a particulate fraction from homogenates by the conversion of labeled ATP to cyclic-AMP.

Major Findings: Corpora lutea varying in age from 2 to 6 days have been assayed for adenyl cyclase activity. The data indicate that there is an increase in activity from days 2 to 6.

Ratios between the control and fluoride stimulated activities and between the LH stimulated and fluoride stimulated ones do not show any consistent change with age of the corpora lutea. This finding suggests that, if there is a special LH responsive type of adenyl cyclase, it varies with age of the corpus luteum in the same manner as the non-specific type. Perhaps a more likely explanation is that only one type of adenyl cyclase is present in corpora lutea and that it is responsive to LH.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the biochemical mechanisms involved in corpus luteum function.

Proposed Course: Terminated.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Solid-Phase Radioimmunoassay of Hormones
Contractor: Battelle Memorial Institute
Columbus, Ohio
Money Allocated: $21,165 (FY 70), $21,165 (FY 71)

Objectives: This is a contract to develop solid-phase radioimmunoassay of pituitary and steroidal hormones in plasma. The investigator will purify HCG and utilize it to test a solid-phase system based upon covalent attachment of antibodies to polystyrene tubes. The solid-phase antibody will replace the antisera and the double-antibody used in currently operating methods. Conditions for optimal measurement will be worked out and compared with these techniques. In the case of the steroid hormones, a derivative of the particular steroid will be coupled to an antigenic protein and the antibodies produced by this conjugate coupled to polystyrene tubes.

Major Findings:
(a) Irreversible binding of antisera to a surface. Original work has been done with the binding of I^{131} albumin to polystyrene and polypropylene tubes. Most of the present work deals with glass tubes. To effect attachment, glass tubes were first exposed briefly to α-aminopropyltriethoxy silane (Union Carbide A-1100), incubated with the antibody and then reacted with glutaraldehyde. In this reaction sequence an amine group is covalently attached to the glass surface through reaction with the amino silane compound. This amine can then be coupled with free amine groups on the protein molecules by reaction with glutaraldehyde.

(b) Reproducibility of the quantity of antisera bound per unit area. The investigator has observed that by limiting the amount of antiserum available for binding he could achieve uniform distribution of antiserum among glass tubes and within the separate sections of the same tube.

(c) Retention of antiserum specificity. In attaching the antiserum to the glass surface care must be taken not to block the antigen reactive sites. Glass capillary tube surfaces appear to have a number of desirable features for antibody attachment, including replicate dimensional characteristics, variety of available sizes, and possible advantages in antigen binding rates stemming from the use of a high surface area to liquid volume ratio. It has been demonstrated the antigen is bound to the fixed antiserum.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on radioimmunoassays of hormones.

Proposed Course: Terminated.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Regulation of the Changes in Corpus Luteum Physiology Required for the Establishment of Pregnancy in the Rat
Contractor: Case Western Reserve University
Money Allocated: $53,313 (FY 70), $53,313 (FY 71), $60,312 (FY 72)

Objectives: The proposed experiments are designed to shed further light upon the interaction between pituitary LH and prolactin and a placental luteotrophic hormone in the regulation of the rat corpus luteum. Interaction between these agents is assumed to be responsible for differences between the corpus luteum of pseudopregnancy and the corpus luteum of pregnancy, the latter being characterized by (a) increased size, (b) ability to maintain increased peripheral plasma levels of progesterone and (c) ultimate development of independence, i.e., the ability to function in the absence of continued support from the pituitary placenta. The fundamental hypothesis to be tested is that in the non-pregnant state LH has a luteolytic action, and the addition to the system of the placental luteotrophic hormone converts this action to a synergistically luteotrophic action.

Experiments will be carried out in the pregnant rat on three principal problems: (1) increase in size of the corpus luteum of pregnancy, (2) elevation in plasma progesterone levels, and (3) development of corpus luteum independence in the absence of pituitary and placenta. Methods to be used include various surgical manipulations, progesterone determination by competitive binding assays, and prolactin determination by radioimmunoassay.

Major Findings: The CL "independence" phenomenon is a process of true progesterone secretion which is demonstrable on the 12th day of pregnancy. It is not related to the maximal progesterone secretion rate and is an effect of placental LTH. In the rat there is no apparent change in metabolic clearance rate of progesterone in pregnancy; level of progesterone in serum of castrated pregnant rats is directly related to the dose administered; estrogen does not affect the serum level of progesterone but improves fetal survival. Serum levels of progesterone in progesterone-treated castrated rats, considerably lower than those of pregnancy, are still consistent with pregnancy maintenance provided estrogen is administered. Anti-LH serum has no aborting effect in presence of adequate progesterone treatment. HCG treatment at the time of ovulation shortens pseudopregnancy. Daily LH treatment to term of pregnant rats hypophysectomized and pituitary-autotransplanted on day 4, with progesterone treatment on days 4 to 11, improves fetal survival over that obtained with progesterone treatment alone. Serum levels of progesterone as high as those of pregnancy occur during lactation; decidualization, or hysterectomy, also increases these levels above those of pseudopregnancy, almost to the levels found during pregnancy. A radioimmunoassay for prolactin has been set up. It does not measure placental LTH.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of

the Contraceptive Development Branch to support research on corpus luteum physiology.

Proposed Course: This is a continuing contractual effort and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of a Laboratory Screening Procedure for Inhibitors of Progesterone Synthesis
Contractor: Harvard University
Money Allocated: $45,120 (FY 70), $45,500 (FY 71), $67,632 (FY 72)

Objectives: The objective of this contract is to define and evaluate agents which interfere with the enzymatic synthesis of progesterone in the corpus luteum. Specifically, the contract concerns the possibility of finding substances which are specific inhibitors of the mitochondrial system which converts cholesterol to pregnenolone; specifically, substances which act at the cytochrome P-450 site. It is suggested that substances exist which act specifically at this site and are non-toxic; such substances would be potential antifertility agents.

The immediate objective of this contract is to devise a convenient and relatively rapid screening procedure which will enable the prediction and preparation of substances which would be biologically effective for the termination of early pregnancy.

Major Findings: The special spectrophotometer to be used in this research has been finally delivered and is operative. It has speeded up the work on changes in the spectrum of cytochrome P-450 enzymes produced by inhibitors of side chain cleavage of cholesterol.

Inhibitors of mitochondrial cholesterol oxygenase (cytochrome P-450-linked cholesterol side-chain cleavage enzymes(s) produced Type II difference spectra with maxima ca. 420 nm and minima at 396 nm and decreased steroid-induced spectral changes when added to bovine corpus luteum mitochondria (BCLM) or solubilized BCLM cytochrome P-450. Both the biological potency as assessed by inhibition of pregnenolone synthesis *in vitro* and the spectral potency as determined by spectral change were closely related. Increasing spectral potency of different classes of inhibitors followed the order: hydrazides, anilines, glutarimides, imidazoles, and pyridine derivatives. Inhibitory potency was associated with low polarity, molecular weights similar to cholesterol and the presence of primary or secondary amine groups. Removal or substitution of the amino group markedly reduced or abolished inhibitory activity. Analysis of extensive series of derivatives indicates that the position of the functional group on the five or six-membered ring of the inhibitor is of considerable importance in the inhibition of cholesterol oxygenase. Study with aminoglutethimide has shown oxygenase. Study with aminoglutethimide has shown that inhibition of progesterone plasma levels can be achieved; however, abortion did not take place.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support development of laboratory screening procedures.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Quantification of Prostaglandins
Contractor: The Upjohn Company
Money Allocated: $42,913 (FY 71), $40,000 (FY 72)

Objectives: The objective of this contract is to develop a radioimmunoassay to measure prostaglandins and certain of their biologically active analogs, and subsequently to quantify these compounds in some body fluids and tissues.

Initially, prostaglandin E_2 and F_2 will be conjugated to bovine serum albumin and polylysine, then injected as a suspension with Freund's complete adjuvant into sheep, rabbits and guinea pigs to produce antibodies to the prostaglandin molecules. The affinity and specificity of the antibody will be characterized by comparing the competitive replacement of radiolabeled prostaglandin by several structurally related prostaglandin analogs. Conditions of incubation, methods of separation, and other alterable variables will be examined to optimize the sensitivity and precision of the assay. In addition, a limited number of prostaglandin analogs with biological activity that indicates a potential clinical advantage over natural compounds will be utilized similarly for assay development.

Circulating levels of prostaglandins will be quantitated during parturition in humans and at various stages of the menstrual cycle in monkeys. Absorption of prostaglandin from various formulations will be followed by quantitating prostaglandin in peripheral serum by radioimmunoassay. In vivo biological effects of administering antisera to prostaglandins will be determined.

Major Findings: A radioimmunoassay for prostaglanding $F_2\alpha$ was successfully developed. Prostaglandin $F_{2\alpha}$ was conjugated to bovine serum albumin, then injected as a suspension with Freund's complete adjuvant into a goat, rabbits and guinea pigs to produce antibodies to the prostaglandin molecule. The affinity and specificity of the antibody were characterized by comparing the competitive replacement of radiolabeled prostaglandin by several structurally related prostaglandin analogs. Conditions of incubation, methods of separation, and other alterable variables were examined to optimize the sensitivity and precision of the assay. Success and utility of this assay system is attested to by the sensitivity (nanogram picogram) rapidity of performance (up to several hundred samples may be determined by one technician per week), and specificity of the assay (small differences in molecular structure can be detected). This range of sensitivity enables one to determine concentrations in peripheral samples of serum and in small amounts of tissue.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on radioimmunoassays of prostaglandins.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: A Model for Studying Inhibition of LH Action on the Corpus Luteum
Contractor: Mayo Clinic and Mayo Foundation
Money Allocated: $36,924 (FY 71), $57,520 (FY 72)

Objectives: A model system has been developed for studying the interaction of human luteinizing hormone (LH) with cells of the corpus luteum using slices of pseudopregnant rat ovaries. The object of this contract is to further validate this model, to define the structural requirements of the LH molecule, to define the requirements of the luteal cell for this interaction, and to seek inhibitors of this interaction. If such inhibitors are found, they may be highly specific abortifacients or contraceptives.

Effort will be made to relate the interaction of LH and the luteal cell to the steroidogenic action of LH. This will be done by assessing the stimulation of estrogen and progesterone synthesis using specific radioimmunoassays. The applicability of this model to human corpus luteum tissue and to the Leydig cells of the male will be investigated.

Major Findings: A model has been devised to study the interaction of LH with corpus luteal cells. Slices of pseudopregnant rat ovaries accumulate LH, FSH, TSH, and insulin. The uptake of radiolabeled LH was competitively inhibited by unlabeled LH and HCG, but not by FSH, TSH, GH or prolactin. This observation can be used as an assay for LH. Binding is temperature dependent, but probably does not require oxidative energy. The binding can also be demonstrated with free cell suspensions and with homogenates of luteinized ovaries. Ultracentrifugal fractionation of luteal cells that have accumulated LH indicate that the major binding is associated with the 110,000 x G pellet. Preliminary autoradiographic studies also suggest an intracellular location of the bound LH. Nonetheless, it has been demonstrated in this laboratory, with this system, as well as in other laboratories using other systems, that LH does activate adenyl cyclase, which is a plasma membrane phenomenon. A paradox exists, however, in that adenyl cyclase activation in unprimed rats is as in rats primed with HCE, while binding of LH is greater in the primed than the unprimed rats. Preliminary data indicate that the LH receptor is protein in nature and that there are structural requirements of the LH molecules for binding.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research in the relationship between LH and the function of the corpus luteum.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of an Automated Immunoradiometric Assay System
Contractor: Veterans Administration Hospital
Palo Alto, California
Money Allocated: $59,110 (FY 71), $30,532 (FY 72)

Objectives: The objective of this contract is to establish a simple automated immunoradiometric assay device, capable of measuring low concentrations of a wide variety of antigens. The assay device is made possible by the prior development (by the applicant) of a new type of immunological assay system (the immunoradiometric assay). In this method, purified radioactive antibodies are used as reagents to convert all the unknown antigen into a directly detectable complex. Unreacted antibody is removed by reaction with an insoluble antigen immunoadsorbent. An important but parallel part of the project is the investigation and development of alternative antibody labels.

Major Findings: The automated immunoradiometric assay apparatus has been put together, and the investigator is testing the mechanical operation of the system.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the development of new types of immunological assay systems.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Effect of Prostaglandins on Ovarian Function
Contractor: University of Miami
Money Allocated: $115,909 (FY 71) for two years

Objectives: The objective of this research is to study the in vivo effect of prostaglandin $F_{2\alpha}$ on the corpus luteum of the menstrual cycle in women in order to determine what acute effects this compound has on the corpus luteum and to determine if it can be used as a luteolytic agent in humans. The volunteer subjects will be divided into two groups. The first group of normal healthy ovulating non-pregnant females will receive an infusion of prostaglandin $F_{2\alpha}$ at different times in their secretory phase of the cycle. This will be considered the control group. The parameters for corpus luteum function will be the measurement of plasma progesterone in the peripheral venous blood. The second group will consist of patients already scheduled to undergo abdominal gynecologic surgery for non-endocrine disease. Their surgery will be scheduled at a time a corpus luteum of a certain age can be expected. Prostaglandin $F_{2\alpha}$ administration will begin at varied times before surgery and progesterone will be measured in peripheral venous blood and in blood obtained from both ovarian veins. In addition, steroidogenesis of incubated luteal tissue will be measured in vitro.

Major Findings: Administration of $PGF_{2\alpha}$ during the luteal phase of the menstrual cycle was without effect on blood progesterone levels or length of the cycle. In several patients, spotting occurred during the course of $PGF_{2\alpha}$ infusion.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the effects of prostaglandins on the corpus luteum.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Molecular Biology of the Human Corpus Luteum
Contractor: New York Medical College
Money Allocated: $56,436 (FY 72)

Objectives: The objective of this research project is to investigate the normal luteolytic process that the CL of the menstrual cycle undergoes and the factors which control it. The investigator hopes that this research will answer a number of important questions among which are:

1. Does progesterone secretion decrease before or after some other fundamental change in the cellular biology of the corpus luteum (CL).

2. Is there a single molecular event which triggers luteolysis, such as an abrupt decrease in RNA synthesis or an abrupt decrease in enzyme synthesis.

3. If there is such an abrupt event, when in the life history of the CL does it occur.

4. Does the CL of the menstrual cycle become the CL of pregnancy simply by preventing the postulated mechanism.

5. Since human chorionic gonadotrophin (HCG) is probably the messenger for the above transition, what is the difference between the effects of human pituitary luteinizing hormone (HLH) and HCG on the molecular biology of the human CL.

6. What is the relationship between the secretion of progesterone by the human CL and the secretion of other steroids which are known to be synthesized by human luteal tissue, especially estradiol.

Significance to Biomedical Research and Program of the Institute: Research on the human corpus luteum is directly related to the stated objectives of the Contraceptive Development Branch.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Study of Polyamines and Ornithine Decarboxylase as a Potential Contraceptive Measure
Contractor: Worcester Foundation for Experimental Biology
Money Allocated: $30,143 (FY 69), $33,740 (FY 70), $16,869 (FY 71)

Objectives: The overall purpose of this program is to establish the primary factors which regulate the metabolism of the polyamines in the reproductive tract, to investigate the role of these substances in the development and growth of the reproductive tract and to determine whether impairment of ornithine decarboxylase activity is a viable means of preventing ovulation.

Major Findings: In screening the tissues for decarboxylase activity, it was found that the ornithine decarboxylase activity of the ovaries showed a sharp rise and decline on the evening of proestrus. Subsequently, it was observed that this stimulation appeared to be under LH control. This enzyme has now been reported in the chick oviduct, rat uterus, prostate and ovary and, in every instance, appears to be under hormonal control.

In the pregnant rat, the investigator observed:

1. Ornithine decarboxylase develops steadily in the fetal placenta until term.

2. At term, 90 percent of the ornithine decarboxylase activity of whole placenta is contained in the fetal placenta.

3. At term, 75 percent of diamine oxidase activity of whole placenta is found in the maternal side.

4. Histidine decarboxylase activity of the placenta is low throughout pregnancy. These observations provide strong evidence that the substrate for high diamine oxidase activity found during pregnancy is, in fact, putrescine.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: Terminated. The study is presently supported by NICHD grant.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Effect of Progesterone and Estradiol on RNA Synthesis in the Rabbit Oviduct and its Relationship to Early Embryonic Development
Contractor: University of Colorado Medical Center
Money Allocated: $26,197 (FY 70), $26,197 (FY 71), $32,344 (FY 72)

Objectives: The objective of this investigation is to study RNA synthesis as a function of hormonal environment in both the embryo and the oviduct during the early stages of environment in the rabbit. Embryos, produced following superovulation, will be transferred to host females whose hormonal status will be altered by endocrine ablation, and estradiol and progesterone administration. The effects of these hormones on implantation, uridine incorporation into RNA, and the type of RNA synthesized will be determined in both the embryo and the oviduct.

Major Findings: RNA synthesis as measured by the incorporation of ^{3}H-uridine is measurable in fertilized eggs, but is very low in unfertilized eggs.

After the two cell stage, uridine incorporation increases slowly with a sudden rapid increase occurring at about 60 hours of development. During the 4-5 day blastocyst stage incorporation remains high but on a per-cell basis, incorporation is somewhat decreased. During the first 48 hours uridine incorporation is random over the nucleus. At 72 hours of development, nucleoli have matured to a reticulated form and uridine incorporation is highly localized over these organelles. RNA synthesis (uridine incorporation) is not detectibly different in embryos raised in vitro through morula and early blastocyst stages. The rate of development of these embryos lags by about 8-10 hours but the sequential pattern of RNA synthesis is not altered.

During early blastocyst stage, a marked increase in protein synthesis occurs. Short pulses with labeled amino acids result in a concentration of grains over the nucleous. This suggests that many of the proteins being synthesized at this time are ribosomal proteins.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the hormonal control of intra-cellular processes.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Ultrastructural and Cytochemical Studies of Trophoblast
Contractor: University of Houston
Money Allocated: $18,829 (FY 69), $29,571 (FY 70), $29,571 (FY 71)

Objectives: The main objective of this research is to study, at the ultrastructural level, the early stages of development and implantation in the rabbit. Although the work is primarily descriptive, some cytochemical methods are being employed to evaluate the role of lysosomes in implantation and in the invasion of the maternal tissues by the syncytial trophoblast.

Major Findings: The dense crystals seen in the rabbit trophoblast have been shown to have proteolytic activity and most likely represent modified lysosomes.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to elucidate the process of implantation and investigate ways in which it may be altered for the purpose of fertility regulation.

Proposed Course: Terminated

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Physiology of Ovulation
Contractor: Trinity University
Money Allocated: $50,005 (FY 69), $51,493 (FY 70), $18,526 (FY 71)

Objectives: The objective of this continuing contract research is to study in mammals the processes related to the phenomenon of ovulation. Although a number of techniques are being employed in these studies, electron microscopy is being emphasized in an ultrastructural examination of ovarian changes preceding and during ovulation.

Major Findings: A variety of chemicals which could be involved in the disruption of follicular connective tissue during ovulation were incubated with strips of sow follicles to determine their effect on the tensile strength of the follicle wall. Commercial preparations of collagenase, elastase, protease, trypsin and chymotrypsin caused significant reduction in the tensile strength of sow follicles in vitro. In addition a series of non-enzymic agents were tested and ascorbate and diketogulonate were shown to reduce tensile strength.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch to support research devoted to elucidating the mechanisms responsible for ovulation and potential means of altering these mechanisms for the purpose of fertility regulation.

Proposed Course: Terminated in favor of a NICHD research grant.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch

Contract Title: Meiosis and Fertilization of Human Ova In Vitro
Contractor: Albert Einstein College of Medicine
Money Allocated: $37,301 (FY 69), $38,918 (FY 70), $18,219 (FY 72)

Objectives: This research is designed to: (1) improve methods for the recovery of ova from women and for their maintenance in vitro; (2) determine the time sequence of the meiotic events of the ovum after its removal from the ovarian follicles; (3) study chromosome morphology and behavior during in vitro and study the cleavage stages.

Major Findings: Ova recovered from women that have previously used oral contraceptives undergo maturation when cultured in vitro. Among such ova abnormal meiosis resulting in diploidy is more frequent than in women that have not used oral contraceptives. In abortuses from women that have a history of oral contraceptives usage the incidence of triploidy is increased.

Significance to Biomedical Research and Program of the Institute: These studies are relevant to the general area of contraception since the production of fertilized human ova in vitro appears the only way to study the physiology and pharmacology of the preimplantation stages.

Proposed Course: The contractual effort is shifted to FRME Branch.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Role of Cyclic AMP in the Action of Estrogen, Progesterone and Relaxin on Uterine Metabolism
Contractor: Emory University
Money Allocated: $24,427 (FY 69), $27,700 (FY 70), $14,487 (FY 71)

Objectives: This investigation is concerned with the relationship of estrogen, relaxin, and progesterone, alone or in combination with cyclic AMP, and the role of the latter, in the regulation of uterine metabolism during various phases of the ovarian cycle.

Major Findings: Uterine phosphorylase activity has been measured after treatment with various hormones and during the estrus cycle. Results indicate that treatment of ovariectomized rats with estrogen increases phosphorylase activity in the resting uterus and that phosphorylase activity in the resting, unstimulated rat uterus increases during the estrus cycle at those times when estrogen secretion by the ovary is known to be increased. Progesterone tends to reverse the effects of estrogen.

Significance to Biomedical Research and Program of the Institute: The metabolic studies undertaken in this project are directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: Termination in favor of a NICHD grant.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Blastocyst Expansion and Pre-ovulatory Follicle Swelling
Contractor: University of Pennsylvania School of Veterinary Medicine
Money Allocated: $23,928 (FY 69), $28,697 (FY 70), $44,539 (FY 71)

Objectives: The objective of this research project is to investigate a much neglected area of reproductive physiology: the mechanisms provided for the accumulation of fluid inside the expanding blastocyst and within the pre-ovulatory follicle. The objective ultimately is to find ways to influence these phenonema as a means toward fertility regulation. The specific projects undertaken are aimed at determining whether or not active transport of sodium, potassium or chloride ions occurs during these rapid periods of growth. This is being done by determining transmembrane potential; ionic flux studies using radioactive calcium, sodium, and chloride; the determination of short circuit current and its correlation with ionic flux; and finally the influence of hormones and drugs on the blastocyst and follicle expansion and in vivo trials with promising compounds. All work is done either in vivo or in vitro with the rabbit.

Major Findings: Using radioactive Na and CL the investigator has demonstrated that there is a unidirectional flux of these ions into the blastocoele. Addition of Cu ions to the blastocyst bathing fluid progressively decreases the short circuit current and potential difference. Copper ions therefore appear to have an inhibitory effect on active transport of ions across the trophoblast wall.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: Renewal not requested. This research will be supported by a NICHD grant.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Role of Cortical Granules in Fertilization
Contractor: Mount Sinai School of Medicine
Money Allocated: $59,389 (FY 69), $44,532 (FY 70), $22,266 (FY 71), $9,401 (FY 72)

Objectives: Isolated cortical granules from homogenates of sea urchin, fish, and frog eggs, are being characterized chemically; and their behavior in the presence of acrosomal extracts and agents that induce parthogenetic development assessed. The initial emphasis on echinoderm eggs was dictated by the necessity of having large numbers of eggs for differential centrifugation. The studies will be extended to mammalian eggs using histochemical methods in place of isolation and chemical analysis.

Major Findings: Intact cortical granules have been isolated from homogenates of unfertilized sea urchin eggs and purified approximately 25 fold. The granules were shown to be rich in acid phosphatase activity. In addition, the enzyme B-1,3-glucanase was shown to be associated with the cortical granule compartment in the unfertilized sea urchin eggs. Half of the glucanase activity in the eggs is secreted into surrounding sea water during the first three minutes after fertilization which corresponds directly to the time when the cortical reaction is taking place.

Cytochemical studies of smear preparations indicated that almost all the particles visible in the cortical granule fractions stained metachromatically or contained sulfated acid mucopolysaccharides. Results indicate that sulfated acid mucopolysaccharides in the cortical granules of the egg play an essential role in fertilization and in establishing the block to polyspermy. These sites have been identified as potentially useful targets for antifertility effects of quaternary ammonium drugs. Pilot experiments designed to test this hypothesis are currently in progress using rats. The data suggest that CTMAB (cetyltrimethyl-ammonium bromide), CPC (cetylpyridinium choloride), and TEAC (tetraethylammonium chloride) were effective in reducing fertility.

Significance to Biomedical Research and Program of the Institute: Little is known about the composition and enzymatic properties of cortical granules and as a fundamental study the proposed work has real scientific merit. It is also related to the contraceptive development program. The true significance of these granules in mammalian eggs is not yet known.

Proposed Course: Terminated

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Control of Ovulation and Capacitation in Non-Human Primate
Contractor: Michigan State U.
Money Allocated: $24,351 (FY 69), $18,100 (FY 70), $26,314 (FY 71)
$42,648 (FY 72)

Objectives: The objectives of this research project are: 1) to develop a reproducible technique for induction of ovulation in primates; 2) to determine the fertilizable life of primate ova; and 3) to carry out further studies on the need for sperm capacitation in the primate.

Major Findings: The investigator has perfected a technique for ovum aspiration utilizing a laparoscopic technique. This technique has been utilized for collection of ova from both M. fascicularis and S. sciureus. In vitro fertilization of ova from S. sciureus has been attempted in culture. Sperm penetration of the zona has been observed; however, presence of two pronuclei or cleavage has not been observed. Thus, fertilization in vitro has been equivocal.

Extensive observations have been made on the morphology of the developing follicles, prior and post-ovulation in M. fascicularis. The preovulatory follicles exibit a particular vascular pattern which is absent in S. sciureus. The ovarian size increases by about 35% prior to ovulation.

The most significant post-ovulatory changes were found to be, a) a generalized hemorrhagic appearance; b) a loss of definition of the follicular vasculature; c) the progressive occlusion of the stigmata; d) a flattening, or irregularity of the follicular wall; and e) partial luteinization. These characteristic changes could be observed as early as 10 hours following ovulation, but the corpus hemorhagicum generally could not be reliably distinguished from the preovulatory follicle until 2 to 4 hours following ovulation. In the majority of the cases the follicular vasculature remained intact through the remainder of the cycle.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies on the necessity for capacitation in primates.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Antigenicity and Immunology of the Trophoblast and the Potentials for the Use of Anti-Trophoblast Serum as A Contraceptive
Contractor: University of Michigan Medical Center
Money Allocated: $38,229 (FY 70), $56,586 (FY 71)

Objectives: When mouse blastocysts are placed beneath the kidney capsule of irradiated hosts, a considerable proliferation of the trophoblast occurs. By combination of the organ culture grid method and the monolayer technique, an almost pure culture of trophoblast cells can be obtained. These cells can be harvested and injected into rabbits and anti-mouse trophoblast serum obtained. Injection of this serum into pregnant mice rapidly causes abortion at all stages but liver necrosis and chronic nephritis develops in the females. However, if the antiserum is fractionated on a DEAE column, a fraction can be obtained which causes abortion but does not affect the liver and kidney.

The objective of this research project is to determine the feasibility of applying this technique to the rhesus monkey. Essentially, these same techniques will be used to prepare rhesus monkey trophoblast antigen and to produce rabbit anti-monkey trophoblast antibody. It will then be established that purified anti-trophoblast antibody is capable of producing abortion reliably in the monkey, and that remating of the monkey is possible without delayed cellside effects of this treatment on reproductive and non-reproductive tract tissues and organs will also be determined.

Major Findings: Treatment of pregnant monkeys with monkey antiplacental antibodies produced abortion in approximately 50 percent of the animals. Another 20 percent could be aborted by a second injection of the antibody.

Proposed Course: Termination.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Studies on the Acrosomal Enzymes Effecting Penetration of the Zona Pellucida
Contractor: University of Pennsylvania School of Medicine
Money Allocated: $28,250 (FY 70), $37,193 (FY 71)

Objectives: In previous work, this investigator and his colleagues have identified a trypsin-like enzyme in the acrosome effecting penetration of the zona pellucida. In their system *in vitro* fertilization could be prevented by use of trypsin inhibitors. The objective of this project is to carry out additional research on the nature and number of enzymes present in the acrosomal complex. An effort will also be made to localize the trypsin-hyaluronidase complex to the inner membrane of the acrosomal cap employing ferritin-labeled antibodies and inhibitors together with electron microscopy. Finally, a search will be made for naturally occurring specific inhibitors of the zona-lysing enzymes in the belief that such inhibitors might be effective antifertility agents.

Major Findings: The trypsin-like enzyme extracted from sperm acrosomes has been given the name "acrozonase." This enzyme has been observed in subcellular fractions of acrosomes from rabbits, rhesus monkeys and men. This enzyme occurs together with hyaluronidase in a 59,000 molecular weight complex. Using florescein-labelled trypsin inhibitors and counterstaining, the localization of "acrozonase" in the acrosome has been confirmed. Epididymal, ejaculated and capacitated rabbit sperm could be stained by the inhibitor.

In vivo fertilization of rabbit ova could be inhibited by injecting the females after coitus with p-amino-benzamidine which is a specific inhibitor or "acrozonase."

Proposed Course: Renewal not requested. Future studies are covered by NICHD program project grant.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Mammalian Oocyte Maturation and Fertilization In Vitro
Contractor: University of Pennsylvania
Money Allocated: $39,945 (FY 70), $41,533 (FY 71)

Objectives: The objective of this research project is to develop an in vitro system for maturation, fertilization, cleavage, and development of the fertilized mouse egg to the blastocyst stage; such blastocysts will then be transplanted into a foster mother to obtain living young. In vitro culture of mouse ova from the 1-cell stage to the blastocyst has already been achieved, but considerable work remains to determine the role of the follicle cell in maturation, the possible dependence of oocyte maturation on hormones, and reproducible conditions for in vitro fertilization. These aspects of the problem will be attacked systematically by culture of oocytes within intact follicles, addition of follicle cells to medium of isolated ova, and addition of amino acids, cofactors, hormones, etc. to the medium.

Major Findings: Addition of serum to the oocyte culture medium improved fertilizability of ova from which cumulus cells were removed.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support the development of culture techniques for the in vitro investigation of ovum-follicle interrelationship, and the hormonal dependence of ovum maturation and fertilization.

Proposed Course: Renewal not requested. Future research in this area is covered by NICHD grant.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Studies on the Antigenic Status of the Ovum
Contractor: Philadelphia College of Pharmacy and Science
Money Allocated: $24,730 (FY 70), $29,056 (FY 71)

Objectives: The objective of this research project is to obtain information on the development of antigens in the mouse, especially the strong histocompatibility-2 (H-2) antigen. The importance of the histocompatibility loci in adult mice, rats and man has been established. Relatively little, however, is known about these antigens during development. In these studies, the investigator will determine the time at which the H-2 antigen appears in preimplantation mouse embryos, and the presence of "embryo-specific" and other transplantation antigens in early embryos. Two-cell mouse embryos will be grown in vitro and the effects of specific allo-antisera on embryonic and trophoblast cells studied during the first eight days after escape from the zona and attachment to the slide. By these methods and immunofluorescence techniques, the precise time of appearance and cell localization of antigens will be determined, as well as the effects of antisera on embryo development in vitro.

Major Findings: Preimplantation embryos at the blastocyst stage have been cultivated in vitro and induced to "implant" on glass coverslips. After treatment with specific H-2 directed alloantisera, there is evidence of immunofluorescence, as reported earlier. However, the technical difficulties of distinguishing between trophoblast and embryo have not yet been solved. In a further attempt to recognize antigenic cells, a more sensitive technique, the mixed hemadsorption technique, is currently being explored.

There is a strong suggestion that 7 1/2 - 8 day embryonic mouse tissue dissected free of the ectoplacental cone can absorb H-2 agglutinins. Confirmatory evidence of this is being sought by means of typan blue exclusion cytotoxicity tests on this tissue.

Highly immunigenic MCA induced tumors are being used to produce tumor antisera. The first sera are ready to be tested. Similarly, antibody to SV40 transformed cells is being prepared, but due to technical problems is not yet ready.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the potentially unique antigenic status of the developing embryo.

Proposed Course: Renewal not requested. Future work covered by a NICHD research grant.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: In Vitro Fertilization of Primate Ova
Contractor: University of Pennsylvania
Money Allocated: $48,740 (FY 70), $55,777 (FY 71)

Objectives: The primary objective of this research project is to develop a reproducible procedure for in vitro maturation of human oocytes and fertilization of ova. The investigator has a successful procedure for in vitro fertilization of rabbit ova and will now extend this experience to the rhesus monkey and man. Ova will be recovered from the oviducts of monkeys and exposed to a suspension of washed sperm or sperm incubated in utero in mid-cycle monkeys. It is expected that these studies will soon indicate whether capacitation of sperm in the female tract is necessary in the primate.

It is also planned to repeat published observations on maturation of monkey and human ovarian oocytes and their fertilization in vitro. The potential for continued development of oocytes matured in vitro will be assessed by transferring them to oviducts of monkeys previously inseminated. They will be recovered later and examined for evidence of in vivo fertilization.

In parallel with these studies, investigations on defining more exactly the biochemical and physical conditions for in vitro fertilization in rabbits will be continued.

Major Findings: Recent efforts have been directed toward identification of early stages of human fertilization and proof of involvement of the sperm cell in this process. Time lapse microcinematographic observations have revealed completion of ovum maturation as evidenced by extrusion of the first polar body 30 hours after recovery of an ovum from a mature follicle of mid-cycle ovarian tissue. By the same method, sperm penetration through the zona pellucida of a human ovum was documented. In the latter instance, the oocyte was cultured for 24 hours to allow completion of the maturation process after removal from a mature ovarian follicle, then inseminated with washed ejaculated human sperm. Sperm penetration through the zona pellucida took place 3 hours after in vitro insemination. Assuming normalcy of the ovum in this experiment capacitation of the sperm, if required, occurred under experimental influences without prior incubation in the uterus of a mid-cycle monkey as was previously done.

Conditions for the in vitro fertilization of rabbit ova have been improved so that an almost 80% fertilization rate is achieved. A greater proportion of ova can usually be fertilized in vitro, when the ova are recovered from the ovarian surface rather than when the ova are recovered from the oviducts of the same ovum donors at the same time.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies on the development

of in vitro systems for the investigation of oocyte maturation and fertilization under controllable conditions.

Proposed Course: Renewal not requested. Future research in this area is covered by NICHD program project grant.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Mechanism of Follicular Rupture
Contractor: Columbia University College of Physicians and Surgeons
Money Allocated: $28,300 (FY 70), $14,150 (FY 71)

Objectives: The objective of this research project is to examine the mechanism of follicular rupture and to determine the feasibility of modifying this mechanism as a method of fertility control. Specifically, the investigator will attempt to isolate the enzyme responsible for rupture of the follicle, identify the enzyme, purify it, and prepare derivatives. By the use of these derivatives or inhibitors of the enzymes, the investigator will attempt to influence ovulation.

Major Findings: Experimental evidence obtained to date indicates that an 0.5% solution of pronase can be used for the isolation of follicles. Liquor folliculi exhibits collagenase activity. Follicles grown in vitro and then ovulated produce changes in the gells on which they are grown.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the mechanism of ovulation and its possible alteration for the purpose of fertility control.

Proposed Course: Terminated.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Studies of the Estrogen-Binding Protein (Estrogen Receptor) in the Human Uterus and Anterior Pituitary and in the Rodent Uterus Anterior Pituitary and the Hypothalamus
Contractor: University of Rochester
Money Allocated: $33,450 (FY 70), $16,725 (FY 71)

Objectives: The objective of this proposal is to compare the molecular properties of the estrogen-binding protein of human uterus and anterior pituitary and of rat uterus, anterior pituitary, and hypothalamus to see whether the receptor substance of one tissue is similar to, or different from, that of another. The receptor proteins will be compared as to sedimentation characteristics, gel filtration chromatography, electrofocusing, binding constant, and the influence of anti-estrogenic compounds on the binding properties.

Major Findings: The data indicate that the estrogen receptor showed similar molecular properties in the rat uterus, anterior pituitary and hypothalamus, with similar ligand specificity as well. The relative quantities of estrogen receptors were different: 560-, 240-, 12 x 10^{-15} moles per mg protein were found in the uterus, anterior pituitary, and hypothalamus, respectively. Using a double reciprocal plot of the competition data, the results indicate that the estrogen and antiestrogens combine with the same site(s) of the receptor in a true competitive manner. The human uterus contains an 8S estrogen receptor with ligand specificity similar to the rat uterine estrogen receptor, but showed a more complex molecular system. The presence of enzymes which alter the molecular size (limited proteolysis) of the estrogen receptor and enzymes which metabolize the estrogens result in a more complex molecular situation.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of steroid binding proteins in reproductive and central nervous tissues.

Proposed Course: Terminated.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Study of Estrogen and Progesterone Binding Substances
Contractor: Mayo Foundation
Money Allocated: $40,960 (FY 70), $41,740 (FY 71)

Objectives: The investigator plans to develop a radioimmunoassay for estradiol, estrone, and progesterone and to measure the concentration of these hormones in arterial blood, in the uterus, and in the uterine secretions during the normal cycle and early pregnancy in the rat. Some observation may also be made in man and correlated with data obtained in the rat. In addition, the investigator plans to develop a radioimmunoassay for the uterine estrogen receptor proteins, to purify and characterize the human cytoplasmic and nuclear estrogen receptor and study their interconvertibility, to study the structure requirements for binding between the receptor and the steroid, and to seek evidence for the existence of a uterine progesterone receptor protein.

Major Findings: As a continuation of the effort to demonstrate that the values obtained by the estrogen radioimmunoassay, which was developed under the contract, truly reflect the gonadal function, the investigators have continued to collect data on normal, hypogonadal and hypergonadal subject. A total of 500 determinations have been performed and the results substantiate the earlier observation. The serum estrogen of normal male and normal fertile female are 4-14 and 4-50 ng percent respectively. Hypogonadal individuals and children have values below 4 ng percent, usually undetectable (below 3 ng percent), patients with estrogen producing tumor showed elevated values.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on estrogen and progesterone binding substances.

Proposed Course: Renewal not requested.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Transfer of Maternal Macromolecules to Mammalian Eggs
Contractor: University of California Medical School
Money Allocated: $29,000 (FY 70), $39,480 (FY 71), $53,075 (FY 72)

Objectives: The principal investigator will examine in detail the macromolecular transfer process in the preimplantation mammalian embryo, working primarily with oocytes and embryos of the mouse and rabbit. There are four general areas of investigation: (1) the composition and origin of mouse and rabbit blastocyst fluid. The blastocyst fluid constituents will be analyzed and the evacuated blastocysts placed in various media. By this means, it is hoped to learn more of the factors controlling the passage of macromolecules into blastocysts. Blastocysts will also be cultured in various antisera to see if they are detrimental to development. If such sera are found, females will be immunized passively or actively to see if development stops in vivo. (2) The composition and origin of rabbit oviductal fluid. Studies will be made of the macromolecular components of rabbit oviduct secretions and these will be compared with the constituents of blood of normal female rabbits. Antisera or analogs will be tested for adverse effects on embryonic development. (3) Electrophoretic studies will be conducted of the enzymes and antigens of the preimplantation mouse embryo. Attempts will be made to identify components which might be blocked to arrest development. (4) Attempts will be made to destroy selectively specific tertiary follicles in the mouse ovary by antifollicle sera.

Major Findings: The investigator has demonstrated that culture of mouse blastocysts and morulae in antiembryo sera was not compatible with further development. Fluorescent antibody studies showed that the antiserum was localized in the zona, and was not detectable in the blastomeres or at the periphery of the blastocyst. Studies on the uptake of ^{125}I-albumin have indicated the following:

(1) Albumin uptake in the absence of additives differs at different embryonic stages, being least at 12 hours, most consistently present at 24 hours and variable at 48 hours.

(2) Albumin uptake is sensitive to the presence of particular additives in the medium, being eliminated by theophylline and DNP and stimulated by cAMP and insulin.

(3) cAMP and insulin differ from each other in their effect on embryonic albumin uptake. In insulin containing medium, uptake is high by 12 hour and 24 hour embryos and varies from no uptake to high uptake in 48 hour embryos. In cAMP-containing media, uptake occurs at all embryonic stages studied but is highest at 24 hours and least at 12 hours.

(4) Metabolic inhibitors in the medium eliminate albumin uptake by the embryo.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on preimplantation mammalian embryos.

Proposed Course: This is a continuing contractual effort and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Uterine Biogenic Amine and Estrogen Relationships, and the Effects of Intrauterine Contraceptive Devices
Contractor: State University of Iowa
Money Allocated: $25,356 (FY 70), $37,492 (FY 71)

Objectives: The investigator plans to study the biogenic monamines norepinephrine, epinephrine, histamine and 5-hydroxytryptamine in the uterus under various physiological conditions. These include cyclic changes during the normal cycle, the effect of exogenous steroids, and the presence of an IUD. Fluorescence histochemistry of biogenic monamines will be used to study monamine localization in relation to (1) uterine blood flow in response to estrogen, (2) the ability of the fertilized ovum to implant, and (3) the effects of an IUD. Individual monamine levels will be manipulated selectively by administration of inhibitors of amine synthesis and the effect of such manipulation determined on uterine response to estrogen and ovum implantation.

Major Findings: Rat uterine mast cells released both histamine and serotonin, as determined by biochemical, fluorescence histochemical and electron microscopic methods, after i.v. injection of a small dose of estradiol; these phenomena are not detectable within the first few minutes after injection, at the time of hyperemia (possibly because of lack of sensitivity of methods). Pharmacological depletion of histamine, serotonin and norepinephrine, complete to the limits of detection of methods, do not interfere with implantation; with the exception of "chemical sympathectomy" with 6-hydroxydopamine (which creates a state of denervation supersensitivity to circulating catecholamines) depletion of amines does not alter the estradiol hyperemic response. Presence of an intrauterine device causes an increase in serotonin and a barely-significant increase in histamine. The IUD will probably cause greater effects if left in place for a longer time.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on metabolic effects of intrauterine contraceptive devices.

Proposed Course: Termination.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Sperm Capacitation as Target for Contraception
Contractor: Vanderbilt University
Money Allocated: $44,278 (FY 70), $44,278 (FY 71), $65,458 (FY 72)

Objectives: The objective of this research project is to perform animal studies aimed at clarifying the role of leukocytes in the capacitation process and improving the methodology for the study of capacitation. Alkylating agents or extracorporeal irradiation of blood will be used to produce systemic leukopenia in an attempt to eliminate leukocytes from the endometrium and uterine lumen. A second objective of the project is the development of a reliable system for in vitro fertilization of human ova.

Major Findings:
Animal Studies: Inhibition of leukocytic response to presence of sperm in female rabbit genital tract indicated that polymorphonuclear leukocytes are not a factor of prime significance in sperm capacitation mechanism. Alkylating agent (nitrogen mustard) used to abolish leukocytic response, however, inhibited capacitation significantly, presumably by interfering with epithelial lining function. Acute early inhibition (within 12-24 hrs) was followed by temporary recovery, followed in turn by slower depression. Inhibition was maximum in uterus, less in oviduct, and even less when both sites were acting sequentially on sperm.

Human Studies: A technique for direct oocyte recovery from any exposed ovary was developed. From 139 oocytes so recovered, 37% were atretic. Of 76 presumably non-atretic oocytes only 23 (30%) completed extrusion of second polar body in culture. Among these, 6 (26%) became fertilized in vitro. In an experiment using oocytes from hormonally primed patients, the addition of FSH, LH and HCG to fertilization medium resulted in significant ($P<.01$) increase in sperm penetrating ability as compared to controls. Such effect is attributed to hormonal stimulation of follicular cells present in fertilization environment. Experimental evidence was thus gained that capacitation is a prerequisite for human fertilization as it is in other mammals.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on new methods of studying complex reproductive functions such as sperm capacitation and in vitro fertilization.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Endocrine Control of Human Oocyte Maturation
Contractor: Peter Bent Brigham Hospital
Money Allocated: $23,213 (FY 71), $40,552 (FY 72)

Objectives: The objective of this contract is to explore a new approach to contraception involving the control of maturation of the human oocyte. Mammalian oocytes are in a resting stage of meiosis until shortly before ovulation. At this time meiosis resumes. The investigators wish to find out what hormones stimulate the resumption of meiosis in humans. They propose to administer these hormones before ovulation in order to induce a premature maturation of the oocyte. They anticipate that if this is done, when ovulation does occur the over-mature oocyte will be incapable of being fertilized.

Major Findings: Follicles were dissected from whole or sections of human ovaries removed at indicated surgery at varying times in the menstrual cycle, and the diameters of the follicles were carefully measured. The follicles removed varied from 2 to 17 mm in diameter. Oocytes were released from these follicles, collected, stained, examined for stages of meiosis, and the stages were correlated with follicle size. The nuclei from follicles up to 6 mm in size were in the germinal vesicle stage. Chromosomes were visible, perhaps in the diplotene stage, in follicles 6 to 8 mm in diameter. First polar bodies were seen in follicles 11 mm in size or greater.

Follicular fluid was obtained from these follicles and other follicles by aspiration. The concentrations of steroid hormones were determined by radioimmunoassay, and the results were correlated with follicular size. On the basis of preliminary findings it appeared that the concentration of estradiol 17-β increased with growth of the follicles while the concentrations did not show any consistent pattern, and it appeared as if the concentration of 17α-OH progesterone decreased in fluid from follicles 7 to 11 mm in size.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on new approaches to contraception involving the control of human oocyte maturation.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Fertilization of Mouse Ova *In Vitro* by Epididymal Sperm
Contractor: The Jackson Laboratory
Bar Harbor, Maine
Money Allocated: $57,700 (FY 72) for two years

Objectives: The investigators have been highly successful in obtaining *in vitro* fertilization (95%) of mouse ova by epididymal sperm. In this contract proposal, it is proposed to define the chemical, physical, temporal, and genetic factors that have contributed to this success.

To the objective of obtaining fertilization in the shortest interval and with the minimum number of sperm, the investigators will study the effect of varying systematically, singly or in combination, ingredients of the medium and other factors of his *in vitro* system; among the important parameters to be studied are the necessity of subjecting epididymal sperm to capacitation, the effect of egg denudation, acrosome reaction, and patterns of attachment of the sperm head to the zona pellucida.

Significance to Biomedical Research and Programs of the Institute: The work to be carried out on this project is directly related to the published purpose of the Contraceptive Development Branch to support research aimed at clarifying factors responsible for successful fertilization.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Hormonal Control of Sperm Maturation
Contractor: Vanderbilt University
Money Allocated: $27,527 (FY 69), $37,083 (FY 70), $50,394 (FY 71), $31,356 (FY 72)

Objectives: This research is directed towards establishing the hormonal factors involved in sperm maturation in the epididymis. The investigator has undertaken a series of endocrine interventions, including ablation and replacement experiments in order to study the effects of these procedures on the fertilizing capacity of epididymal sperm of the male rabbit.

Major Findings: The investigator has continued her studies on the epididymal maturation of spermatozoa, and has used as a model the ligated corpus epididymis. Under normal conditions, the sperm from this region have very low fertilizing ability. In the rabbit 24 hour ligation of the upper corpus region increases the fertilizing ability of sperm from this region to between 60 to 70%. In animals castrated less than three days there was no apparent effect of castration on fertilizing ability. In animals castrated for three or four days, ligation of the corpus did not improve the fertilizing ability of sperm as it did in the one or two-day castrates. Administration of testosterone to castrated rabbits improved sperm motility and fertilizing ability. It is not clear whether the effect was direct on spermatozoa or indirect on the epididymis.

In hypophysectomized male rabbits, the spermatozoa (lower corpus) become infertile after five days. In hypophysectomized rabbits given testosterone replacement therapy from the day of operation the spermatozoa from the lower corpus retained their normal fertility. Testosterone injections initiated seven days after hypophysectomy and continued for seven days restored the fertilizing ability of uterine inseminations but had only a marginal effect on tubal inseminations. Administration of cyproterone acetate to normal rabbits for seven days was without effect on sperm fertility.

In the rat study, the investigator has demonstrated that castration and hypophysectomy both lower the fertility of epididymal spermatozoa. Testosterone can counteract the effects of both castration and hypophysectomy. In the rat, cyproterone acetate counteracts the action of testosterone.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research directed towards development of new, male methods of contraception.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Morpho-Physiologic Studies on Mammalian Gametes Prior to and During Fertilization In Vivo and In Vitro
Contractor: Harbor General Hospital
Money Allocated: $23,783 (FY 69), $42,416 (FY 70), $45,978 (FY 71) $39,714 (FY 72)

Objectives: This work involves light and electron microscope studies of gametes, especially the acrosomal changes in fertilizing sperm and the cortical granules of eggs prior to and during fertilization. These techniques will also be used to determine the number of sperm around mouse eggs at the time of fertilization, and whether it is the first sperm, or another among the many in the vicinity, that fertilizes the egg. Sperm transport in the uterus and the oviduct and egg transport in the oviduct are also being investigated.

Major Findings:

1. Fertilization of mouse eggs in vitro.
   A large number of eggs were fertilized in vitro, fixed and sectioned for examination by EM. Criteria used to assess fertilization were the identification of sperm fragments in the ooplasm in conjunction with occurrence of meiotic division, formation of pronuclei or normal cleavage. The zona pellucida was penetrated by spermatozoa with and without acrosomes which could be seen in the perivitelline space. Identification of "the fertilizing sperm" was extremely difficult.

2. Maturation of human oocytes in vitro. Oocytes were obtained from follicles 0.1 to 1.5 cm in diameter in ovarian wedge sections removed at laparotomy. Following incubation in Ham's F10 alone or with added albumin for 24 to 53 hours, the oocytes were removed and prepared for light or electron microscopy. Subsequent examination revealed that some oocytes could be maintained or even matured in vitro, thus supporting this approach to in vitro fertilization of human ova.

3. Ultrastructural study of monkey oocytes in vitro and in vivo. Contrary to findings with human oocytes, few monkey oocytes attained maturation in vitro and regression involving alterations in the distribution and structural organization of cytoplasmic organelles and nuclear components was frequently observed.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch as it pertains to the mechanisms involved in the critical process of fertilization.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Ultrastructural and Electron Cytochemical Studies of Mammalian Spermiogenesis
Contractor: The University of Texas
Money Allocated: $35,875 (FY 69), $58,230 (FY 70), $64,282 (FY 71), $82,127 (FY 72)

Objectives: The research project involves studies of (1) the formation of the manchette in the spermatids during spermiogenesis, by electron microscopy and cytochemistry at the electron microscopic level (ATPase, succinic dehydrogenase, alkaline and acid phosphatases, thiamine phosphatase, aryl sulfatase and β-glucuronidase); (2) the inhibition of microtubule formation in the manchette (colchicine, cold shock, inhibitors of protein and RNA synthesis); (3) the ultrastructure and cytochemistry of seminal vesicles, bulbourethral gland and prostate.

Major Findings:
1. Experiments with Colcemid and other microtubule inhibitors have resulted in the description of several microtubular systems in cells of the seminiferous epithelium of Chinese hamsters and mice. An effective dose of the drug has been determined which, when injected daily, brings about a broad spectrum of damage to the seminiferous epithelium leading to complete infertility within 35 days. Treated animals remain healthy and sexually active. Animals recover to normal levels of fertility within 75 to 90 days following cessation of treatment. Recent experiments with photochemically inactivated Colcemid provided convincing evidence that testicular damage was due to disruption of microtubules in germ cells and Sertoli cells.

2. Base-line electron microscopic cytochemical studies have been conducted on normal and experimental treated Chinese hamsters. Recent experiments with Actinomycin D have shown that spermatocyte RNA synthesis can be inhibited *in vivo* leading to specific damage to primary spermatocytes.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch regarding the development of new male methods of contraception.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Biochemical Studies on Spermatogenesis and Spermatozoa
Contractor: Northwestern University
Money Allocated: $39,944 (FY 69), $45,061 (FY 70), $45,061 (FY 71), $37,174 (FY 72)

Objectives: The applicant has published extensively on lactate dehydrogenase isozymes. Relevant to the current proposal are his studies on the LDH-X isozyme found in testis and spermatozoa. The applicant is now studying the role of this isozyme in spermatogenesis and the metabolic economy of epididymal and ejaculated spermatozoa, and determining whether other testis-specific forms of other enzymes exist and what part they may play in the biochemistry and function of the male reproductive system. The applicant asks the question, does LDH-X have any functional role in sperm production, motility or functional capacity? His immediate approach to such studies is to isolate sufficient quantities of the LDH-X isozyme to produce antibodies to this enzyme. Mouse testes are used as the source for the enzyme and antibodies are raised in rabbits. These antibodies to the LDH-X and other subunits, e.g., LDH-1 and LDH-5, will be used to isolate LDH labeled during biosynthesis in the animal using radioactive amino acids. From these data, isozyme synthesis rates or isozyme half-life can be calculated.

Major Findings: Progress is reported in the area of (1) LDH-X purification, (2) antiserum to LDH-X production, and (3) antifertility effects of anti-LDH-X antibody.

In the area of LDH-X purification a homogeneous preparation has been obtained in crystalline form. Purity has been established by electrophoresis and by immunochemical homogeneity.

Injection of males with anti-LDH-X antibody produced a reversible loss of fertility. The depression of male fertility was not total.

Injection of the antiserum into pregnant mice produced a lowering of fertility level and resulted in termination of pregnancy in pregnant animals.

Preliminary data indicate a reduction in fertility among rabbit does which were injected with LDH-X for the purpose of antibody production.

Significance to Biomedical Research and Program of the Institute: It is important to establish LDH isozymes turnover rates in the testes since this information is basic to the complete understanding of the normal function of the testes. Studies of this sort are highly relevant to the purposes of the contraceptive development program.

Proposed Course: This continuing contractual effort leading toward the development of new contraceptives is an integral part of a targeted research program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Role of the Epididymis in Sperm Maturation
Contractor: Pennsylvania State University
Money Allocated: $30,706 (FY 69), $58,660 (FY 70), $65,359 (FY 71)
$89,736 (FY 72)

Objectives: The specific objectives are: (1) to determine actual sperm production rate in bulls; (2) to compare ultrastructure and biochemical characteristics of spermatozoa recovered by cannulation of the rete testis with those of cells recovered by cannulation of the vas deferens; (3) to characterize biochemically the testicular effluent and, by comparing this with seminal plasma and accessory gland fluids, to establish the nature of the epididymal secretion; and (4) to determine if there is a substance in the testicular effluent which might exert a local control on spermatogenesis or epididymal function.

Major Findings: Studies on sperm production measured by obtaining daily ejaculates and collecting samples from rete testis or vas deferens cannulae indicate that little sperm resorption occurs in the epididymis.

Testicular sperm appeared to be biochemically, metabolically, and immunologically different from epididymal or ejaculated cells. Testicular sperm contained 2-3x more total phospholipid and 5x more inositol than epididymal sperm. Testicular sperm also utilized endogenous free inositol *in vitro* but this and sperm respiration were depressed by the addition of steroids. A technique for quantitating the amount of fluorescein-labeled immune-globulin bound to different portions of individual sperm has been developed.

Electrophoretic analyses revealed that bovine cauda epididymal plasma contained antigenic substances not detectable in blood serum or in the testicular effluent. The epididymis appears to absorb testosterone found in rete testis fluid, a 200-fold difference being observed in the 24-hour excretion rate monitored at the rete testis and cauda epididymis. Dihydrotestosterone was detected in the cauda plasma but never in the rete testis effluent.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies directed towards the development of new male contraceptives.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Morphological and Experimental Studies on Spermatogenesis, Sperm Maturation, and Means of Inducing Reversible Infertility in the Male
Contractor: Harvard Medical School
Money Allocated: $66,656 (FY 69), $59,944 (FY 70), $68,151 (FY 71), $71,702 (FY 72)

Objectives: This research is centered upon the use of modern, high quality ultrastructural techniques to investigate key issues of testicular and spermatozoal morphology function. One project deals with topics in spermatogenesis. The investigators are studying the significance of the chromatoid body and aggregates of spermatocytes within the seminiferous tubules, electron microscope radiographic studies of RNA and protein synthesis in spermatocytes and spermatids and, by the use of mitotic blocking agents, interfering with specific developmental events in spermiogenesis in order to learn more about the function of micro-organelles found in germinal cells.

Major Findings:

1. Studies on the blood testis barrier. This barrier which consists mainly in specialized junctional complexes between Sertoli cells was found to be established around the 18th postnatal day in the rat. Suppression of gonadotropin by administration of clomiphene or estradiol to new born rats delayed the formation of Sertoli cells junctions up to 35 days when the barrier became established.

2. Correlation of the ultrastructure and pattern of locomotion of spermatozoa. High speed cinematography has shown that a wave of contraction starts at the junction of the mid-piece and principal piece and not at the centriole of the neck piece. The antifertility substance U-5897 was not found to affect sperm motility in contradiction to reports in the literature.

3. Comparative studies of development and ultrastructure of spermatozoa. Observations were made and published indicating that the microtubules of spermatid's manchette have no influence on sperm head's morphology. Instead the shape of the nucleus is determined by agenetically controlled condensation of the chromatin. Further observations were made on ultrathin sections and by freeze-clearing postacrosomal region of human and monkey spermatozoa.

4. Investigation of the site of action of bis (dichloroacetyl) diamines on spermatogenesis. Following the collection of a set of reference EM photomicrographs of the stages of spermatogenesis of the guinea pig the effects of small doses of this compound were analyzed. The earliest alterations were observed in the condensing nucleus, followed by modification of the acrosome. Spermatocytes and Sertoli cells were also affected in various

ways. The analysis of these results did not confirm the postulated role of the manchette or the microtubules of Sertoli cells in shaping the head of spermatozoa.

Significance to Biomedical Research and Program of the Institute: Studies of these topics are deemed highly relevant to the purposes of the contraceptive development program.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Mammalian Sperm Motility and Ultrastructure
Contractor: Washington University
Money Allocated: $42,699 (FY 69), $40,543 (FY 70), $52,589 (FY 71), $70,344 (FY 72)

Objectives: The objectives of this research project are: (1) to study in a variety of species both sperm ultrastructure and sperm motility in an attempt to correlate them, and (2) to study the fine structural differences in spermatozoa before and after capacitation.

Major Findings:

1. Rat sperm exhibit higher beat frequencies after 3.5 hours of uterine sojourn than after 5-15 minutes. Ultrastructural differences accompany the observed differences in motility. Spermatozoa at 3.5 hours post-insemination often possess a dense material which coats the plasma membrane overlying the sperm head. This material is not present on all spermatozoa and is sometimes found associated with plasma membrane in other regions of the cell. The matrix of sperm mitochondria at 3.5 hours post-insemination appears considerably less electron dense than the mitochondrial matrix of spermatozoa at 5 to 15 minutes post-insemination. The change in mitochondrial morphology might be correlated with changes observed in motility.

2. Examination of the motility patterns of squirrel spermatozoa indicates that both asymmetric beating patterns and asymmetric head shape can result in rotation of the sperm and that in some species an asymmetric sperm head is the major factor affecting rotation while in other species the asymmetric beat produces rotation.

3. Preliminary studies on sickle-shaped sperm heads from mice suggested that the hook-shaped head may function primarily in fertilization rather than motility.

4. In slow currents rabbit spermatozoa assume random orientation.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on the correlation of sperm function and ultrastructure especially as it is affected by capacitation.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Fine Structure and Function of the Accessory Glands of the Male Reproductive Tract
Contractor: University of Colorado
Money Allocated: $14,985 (FY 69), $12,750 (FY 70), $18,266 (FY 71), $25,851 (FY 72)

Objectives: This study of the fine structure and function of the male accessory organs is being carried out principally in rats, with particular attention being paid to (1) effects of vasectomy, both proximal and distal, to the point of ligation of the vas; and (2) changes which take place in the Wolffian ducts of rat fetuses, before, during, and after onset of androgen secretion (at daily intervals from day 14 until birth). Electron microscopy and electron microscope radioautography are being used to follow the fate of radio-labeled precursor substances in a study of the secretory activity of the accessory glands.

Major Findings: In the study of post-vasectomy changes in the rat epididymis the investigator has observed alterations in the fine structure of the epididymal epithelium approximately six months after vasectomy. The changes are most pronounced in the so-called "light cells." After bilateral vasectomy, there was an accumulation of membraneous material within membrane-bounded structures in several locations in the cytoplasm of the light cells of the cauda epididymidis.

On some occasions, recognizable parts of sperm were observed in apical vacuoles in epididymal epithelial cells after vasectomy. They were not seen in sham-operated animals or in normal rats.

The large infranuclear structures with a polymorphic interior in the epididymal epithelim are identified morphologically as large lysosomes (deDuve and Wattiaux, 1966). The origin of their contents is not definitely known, but they accumulated most rapidly after vasectomy when it is necessary to account for the disposal of large numbers of sperm.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Biochemical Requirements for Fertilization and Development of Rabbit and Human Ova
Contractor: University of Georgia
Money Allocated: $34,122 (FY 69), $45,351 (FY 70), $46,519 (FY 71)

Objectives: This research project involves the study of at least three enzymes present in sperm, the corona-removing enzyme (CRE) and two trypsin-like acrosomal enzymes which, the applicant hypothesizes, are involved in the penetration and removal of the corona radiata, the zona pellucida and the vitelline membrane. The applicant further postulates that "decapacitation factor" DF) is a low molecular weight material which inhibits the CRE and thus blocks fertilization or capacitation. Both rabbit and human materials are used to study these points. Included as a related objective is the development of an effective medium and the proper physical conditions for *in vitro* fertilization and cleavage of human ova obtained from ovarian tissue removed at local hospitals.

Major Findings: Decapacitation Factor (DF) - The peptide nature of this naturally-occurring antifertility factor was further confirmed. To obtain quantity sufficient for characterization the partially purified protein mixture from bull seminal plasma was digested with chymotrypsin (since both trypsin and Pronase destroy some DF activity) after which the active moiety was dialyzable. Fractionation on cellulose columns yielded an active preparation containing only one major peptide with two minor peptides. The biological DF assay was improved by use of superovulated rabbits as ovum donors and artificial insemination to obtain capacitated sperm. Pronase digests of polylysine and polyarginine have an antifertility effect on capacitated sperm in the DF assay system.

Acrosin, the acrosomal proteinase that digests the zona pellucida of the ovum, possesses unusual specificity. Purified acrosin preparations are highly specific for arginyl peptide bonds with little effect on lysyl bonds and no effect on other amino acyl bonds making acrosin distinctly different from trypsin. Acrosin is markedly inhibited by free arginine. Thus, this amino acid is an excellent model for designing acrosin inhibitors with antifertility action. Tosyl lysine chloromethyl ketone (TLCK) inhibits acrosin and used vaginally in K-Y jelly reduces fertility in rabbits to 21% of the ova fertilized while a "shot gun" mixture of several synthetic and natural proteolytic enzyme inhibitors reduced fertility to almost zero.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch to elucidate the mechanisms involved in sperm capacitation and in the critical process of fertilization.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Metabolism in Spermatogenesis and Sperm Maturation
Contractor: Boston University
Money Allocated: $11,749 (FY 69), $14,770 (FY 70), $22,491 (FY 71)

Objectives: This research project is involved with determining the biosynthetic potential of testicular spermatozoa and of spermatozoa from the caput and cauda of the epididymis by studying the biosynthesis of certain key lipids. It may then be possible to uncover a critical stage of metabolism in sperm maturation which may then be vulnerable to specific inhibitors.

Major Findings: Treatment of mature male rats with a number of progestins resulted in the reduction in weight of accessory sexual organs, inhibition of spermatogenesis and libido. Combined treatment with progestins and androgens restored libido without influencing spermatogenesis.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies directed towards the development of new male methods of contraception.

Proposed Course: Termination.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Formation of the Subacrosomal Mass (Perforatorium) of the Golden Hamster Spermatozoa
Contractor: Tulane University
Money Allocated: $14,451 (FY 69), $15,347 (FY 70), $15,347 (FY 71), $20,047 (FY 72)

Objectives: The investigator is studying the origin of the perforatorium of golden hamsters' sperm by following initially the formation of the perforatorium in early spermatids by electron microscopy. The hamster is chosen because of the large size of the hamster perforatorium; however, these studies are being performed, and chemical characterization of the perforatorium attempted.

Major Findings: Contract studies have resulted in:

1. Identification of the rodent "perforatorium" as an extension of the subacrosomal region (subacrosomal space, apical body) which is an anatomical feature common to mammalian sperm.

2. Demonstration that the morphological circumstances of acrosome production are consistent with the belief that the acrosome represents a special form of lysosome.

3. Demonstration that the apical body (subacrosomal region) has a complex morphogenesis, perhaps involving the nuclear envelope; thus demonstrating that the current widely held assumption that the apical body is merely trapped residual cytoplasm, is incorrect.

4. The studies are directed toward the elucidation of the possible functions of the subacrosomal region in fertilization. To accomplish this, the investigator is preparing antisera to "acrosome-free" sperm and to "acrosomal ghosts."

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purposes of the Contraceptive Development Branch to support research on potential areas of male contraception.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Prostaglandins and Smooth Muscle Responses of Male Sex Organs
Contractor: University of Oregon
Money Allocated: $32,750 (FY 69), $34,744 (FY 70), $35,870 (FY 71)

Objectives: The objective of the project is to compare the effect of the prostaglandins E and F compounds on: (1) the smooth muscle responses in male sex organs (seminal vesicle, vasa deferentia, ductus epididymis seminiferous tubule); (2) the in vitro and in vivo smooth muscle response of the same organs to other smooth muscle stimulants such as adrenalin, serotonin, vasopressin, oxytocin and the neurotransmitters noradrenalin and acetylcholine; (3) the smooth muscle response in male sex organs of normal or castrated animals. The investigation is aimed at the understanding of the sites and mechanism of action of the prostaglandins in smooth muscles.

Major Findings: PGA_1 and $PGF\alpha$ show similar response to PGE_1 in that greater than 1 ug of the prostaglandin is required in the bath for any effect to be evidenced at all. An interesting aspect of response of the vas to PGA_1 and PGF_1 is that the response appears to be all-or-none at its own threshold as well as at higher levels - e.g. if 10 ug $PGF_1\alpha$ will augment a 5 ug NA response with a 1.0 mv increase, a 9 ug $F_1\alpha$ dose may have no effect at all and a 100 ug dose may have no greater effect than a 10 ug dose. The most apparent difference in the PGA_1 and $PGF_1\alpha$ response appears to be in the order of effectiveness. It required about five times the quantity of PGA_1 to achieve a response comparable to that of the threshold amount of $PGF_1\alpha$.

PGE_2 remains the most effective prostaglandin in augmentation of the vas deferens response to adrenalin, noradrenalin, acetylcholine, or transmural electrical stimulation (TMES). In the normal animal immediate responses are noted with as little as 0.05 ug PGE_2 dose in the 10 ml bath when used in conjunction with any of the agonists. Doses at a higher level retain the augmented agonist response over several trials without further PGE_2. Thus, PGE_2 appears to be the prostaglandin of choice in our efforts to elucidate the mode of action of the prostaglandin effect on the smooth muscle of the hamster vas deferens.

Significance to Biomedical Research and Program of the Institute: This project is related to the purpose of the contraceptive development program particularly in its effort to delineate the mechanism of sperm transport in the epididymis.

Proposed Course: Termination. Renewal not requested.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Formation of Dihydrotestosterone in Male Sex Organs and Transport of Steroid Androgens from the Testis to the Epididymis
Contractor: University of Southern California
Money Allocated: $36,000 (FY 69), $45,250 (FY 70), $53,257 (FY 71), $65,188 (FY 72)

Objectives: The contractor is studying epididymal relationships and formation of two androgens, testosterone and dihydrotestosterone. He is developing a method for submicrogram quantification of dihydrotestosterone in a biological sample in order to measure the levels of testosterone and dihydrotestosterone in testicular tissues, spermatic venous blood, spermatic lymph, corpus, and cauda epididymis.

Major Findings: It was demonstrated that 5α-dihydrotestosterone is a secretory product of isolated dog prostate infused with its own arterial blood and that secretion of the 5α-DHT is not influenced by lactogenic hormone. The author demonstrated that both the Mullerian and Wulffian ducts of 20 day old rat fetus convert testosterone to 5α-DHT. Compared to the Wulffian duct, the isolated testis of the unborn rat produces small amounts of 5α-DHT from T, though production of radioactive 5α-DHT from radioactive acetate can be recorded in isolated testes of the 20-day rat fetus. 5α-DHT has been demonstrated to be a secretory product of dog testes and its production has been shown not to be stimulated by administration of HCG. Testicular tissue of adult rats was shown to contain 5α-DHT. Testicular 5α-reductase was shown to be more active in testes of young rats. Observations were made to show that Leydig cells of mature rat testes do not contribute to testicular production of 5α-DHT from testosterone but they appear to do so in young rats. Infusion of testosterone in the testicular artery of the dog induced appearance of 5α-DHT in lymph of the infused organ.

Significance to Biomedical Research and Program of the Institute: This project is directly relevant to the contraceptive program since epididymal physiology has been identified as a particularly important topic for concentrated attention.

Proposed Course: This is a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Immunological Control of Reproduction
Contractor: Tulane University, Delta Regional Primate Research Center
Money Allocated: $23,936 (FY 70), $23,936 (FY 71)

Objectives: This is a contract to investigate: (1) the effects of testicular homogenate and sperm antigens on spermatogenesis in the male and fertility in the female; (2) the effects of homogenates of fetal placental tissue on pregnancy maintenance or conception when given to gravid or non-gravid females, respectively; (3) the effects of rabbit or sheep antiplacental sera on fertility in the female; (4) the characterization of the specific sperm or placental antigens involved in the above three sections. More specifically, during the first year, the applicants will immunize monkeys with autologous and homologous sperm, or testicular homogenates, and evaluate spermatogenesis, testicular reaction, and fertility. During the second contract period, they will repeat this in rabbits and monkeys using placental antigen. During the third phase of the program, they will attempt to identify the antigens and establish the degree of organ specificity of the antibodies formed against these antigens.

Major Findings: Rhesus males immunized with testes and sperm antigens respond with increased serum antibody titers. Aspermatogenesis could not be induced by this technique and testicular morphology remained essentially normal.

Significance to Biomedical Research and Program of the Institute: The immunological studies undertaken in this project are directly related to the published purposes of the Contraceptive Development Branch.

Proposed Course: Due to negative results, renewal of this contract was not requested.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Maturation of Mammalian Sperm in the Male and Female Reproductive Tract
Contractor: Columbia University College of Physicians and Surgeons
Money Allocated: $72,770 (FY 70), $22,998 (FY 71)

Objectives: The first objective of this research project is to study the process of sperm maturation in the male genital tract: (1) by ultrastructural studies of the morphological changes occurring in the acrosome during spermiogenesis in the human; (2) by an investigation of changes in the sperm plasma membrane during epididymal maturation in normal and ligated rabbit epidiymides, and in the normal monkey and human epididymis; (3) by an investigation of the development of sperm motility in the primate and human epididymis; (4) by studies in rats and rabbits of the genetic consequences of spermatogenic inhibition or of disruption of epididymal function during the period of declining fertility in order to assess the risk of abnormal offspring after treatment with antifertility agents.

A second objective is a study in the female of the factors affecting the sperm acrosome reaction: (1) by characterizing chemically the decapacitation factor in bull and rabbit seminal plasma; (2) by studying the influence of the rabbit seminal plasma decapacitation factor on the acrosome reaction of sea urchin sperm; (3) by studying the influence of follicular fluids on the sperm head plasma membrane/acrosome complex of rabbit primate and human spermatozoa; and (4) by studying the structural responses of human and primate sperm to homologous oocytes matured in vitro.

Major Findings:

1. The following changes occur in spermatozoa during their maturation in the human epididymis.

   a. Changes occur in the chemistry of the surface - these, shown in the rabbit also, depend on the epididymal environment. In man, a "mature" surface appears to develop in the lower corpus epididymidis.

   b. Maturation of motility - human spermatozoa showing normal sustained motility seem to first appear in the lower corpus epididymidis. Notwithstanding biochemical changes, this can also be correlated with change in the structural (-S-S-) bonding of the substance of the outer dense fibres of the midpiece and sheath of the tail.

   c. The nucleus of the human spermatozoon undergoes a significant structural stabilization by establishment of -S-S- cross-links between the nuclear proteins during epididymal passage. This occurs in all mammals, but only in man do the sperm nuclei in the ejaculate appear as a heterogeneous population. The nuclei in sperm flushed from the uterus are also not uniform structurally.

2. The study on effects of vasectomy in several species (rabbits, hamsters and rhesus monkey) has indicated that the pressure build-up as the result of vas occlusion produces epididymal rupture. There was no indication of sperm reabsorption in the epididymis.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies devoted to elucidating the factors in the male and female reproductive tract responsible for the various phases of sperm maturation and the development of fertilizing capability.

Proposed Course: Termination. Renewal not requested.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Enzymes of the Sperm Acrosomes, Occurrence of the Inhibitors of These Enzymes in Seminal Plasma and the Role of the Enzymes and Inhibitors in Capacitation of Sperm and in the Penetration and Fertilization of Ova
Contractor: University of Georgia
Money Allocated: $29,494 (FY 70), $45,835 (FY 71), $44,462 (FY 72)

Objectives: The objectives of this research project are: (1) to isolate and purify the five acrosomal enzymes from mammalian sperm; (2) to demonstrate their role in fertilization; (3) to elucidate the role played by enzyme inhibitors in seminal plasma (including decapacitation factor, DF); (4) to attempt to correlate DF activity with the inhibition of a new sperm neuraminidase; and (5) to study uterine enzymes and their possible role in capacitation.

Major Findings:

1. Acrosin has been purified further. The specific activity is now double of what was reported earlier representing about 500-fold purification.

2. Substrate specificity of acrosin has been studied. The results indicate that acrosin is a arginyl protease and is inhibited by arginine. It appears to be a unique enzyme.

3. Attempts have been made to purify acrosin-acrosin inhibitor complex, to isolate the inhibitor from the complex and to determine if this inhibitor is similar to the acrosin inhibitor purified from seminal plasma (SPAI).

4. Sperm neuraminidase (SN) has been purified using affinity chromatography. This purified SN shows two bands on disc electrophoresis and is devoid of acrosin and hyaluronidase activities. Purified SN has the same action on the zona pellucida as previously reported for crude preparations, i.e. SN treatment inhibits fertilization and renders the zona resistant to trypsin dissolution.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies of the role of sperm acrosomal enzymes and their inhibitors in capacitation and fertilization.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: An Evaluation of the Efficacy of Testosterone-Filled Dimethylpolysiloxane Implants to Maintain Varying Concentrations of Testosterone in the Peripheral Circulation of Male Rabbits
Contractor: Oklahoma State University
Money Allocated: $28,917 (FY 70), $14,459 (FY 71)

Objectives: The objectives of this research project are to study: (1) The diffusion of labeled testosterone from dimethylpolysiloxane (DPS) capsules *in vitro* in distilled water in order to establish the appropriate capsule dimensions for a spectrum of 24-hour diffusion rates *in vivo*;(2) the effects of various capsular dimensions on the concentration of tritiated testosterone in the peripheral plasma of male rabbits; (3) the effects of implanting tritiated testosterone-filled DPS capsules into various anatomical sites on the concentration of tritium in the peripheral plasma of male rabbits; and (4) study the efficacy of DPS testosterone implants for releasing and maintaining peripheral testosterone concentrations at some predetermined level for six months in intact and castrated male rabbits.

Major Findings: It has been clearly demonstrated that subcutaneous implantation of testosterone filled DPS implants produces azospermia without depression of libido.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to develop new methods of systemic or local administration of contraceptive agents in both the male and female.

Proposed Course: Terminated.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Control Mechanisms of Sperm Motility
Contractor: State University of New York
Money Allocated: $30,836 (FY 70), $38,170 (FY 71), $34,523 (FY 72)

Objectives: The applicant proposes that sperm motility is the result of a control system consisting of a free-running oscillator, an amplifier, a power supply, and a feedback loop. The investigator proposes a number of novel experiments designed to investigate the nature of this control system and its components. Specifically, he proposes: (1) To measure the electrical activity of the oscillator in the centriole by inserting glass microelectrodes into the head of sperm immobilized first by low temperature, and to search for electrical potentials of the same frequency as the tail wave. (2) To destroy by a laser beam, areas of the centriole. A glass microelectrode will then be inserted into the head of the immobilized sperm to monitor the electrical activity, and to attempt electrically to reactivate the mechanism for motility. (3) To inhibit the action of the oscillator and its coupling, by specific chemicals such as xylocaine and procaine. (4) To investigate the feedback mechanism by inhibiting respiration, and then measuring the change of frequency of the tail wave.

Major Findings: The investigator reports the following basic observations:

1. Impaled sperm have been reactivated by means of ATP and of ADP. The contractile process in these reactivated sperm appears of the normal type.
2. Initial reactivation experiments with human sperm duplicate the results under 1 for bull sperm.
3. The infra red laser has successfully been put in operation. The centriole area appears to be the most sensitive to irradiation.
4. New stiffness measurements have confirmed the previous dynamic ones. The stiffness of a flagellum depends on the ATP and ADP content.

Concerning the subject of sperm motility the investigator has reached the conclusion that the original hypothesis concerning the centriole as the "oscillator" will have to be modified. The reactivation experiments have shown that apparently normal contractible activity can occur without the presence of the centriole and that the contractile system itself is the oscillatory system.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies in which electrophysiological techniques are applied to elucidating complex reproductive processes such as sperm motility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Ultrastructural and Immunological Investigation of Spermatogenesis, Sperm Transport, and Fertilization in the Mammal
Contractor: University of Miami
Money Allocated: $92,300 (FY 70), $58,275 (FY 71), $78,025 (FY 72)

Objectives: The objective of this research project is to study the effects of antibodies on spermatozoa in the female tract of mammals, using bivalent and univalent fragments of guinea pig and goat anti-rabbit semen antibody.

The investigators will examine the fertilizing capacity and transport of antibody-pretreated spermatozoa following intrauterine artificial insemination, in an attempt to establish whether antibodies can inhibit spermatozoan passage from the uterus through the tubes to the site of fertilization. In addition, intratubal and/or in vitro insemination experiments with univalent and bivalent antibody-treated spermatozoa will be performed in an attempt to determine if antibody treatment inhibits capacitation and whether antibody can inhibit the fertilizing capacity of capacitated sperm. Anti-sperm antibody inhibition of sperm head enzymes thought to be responsible for ovum penetration will be tested by preparing extracts of these enzymes and examining their activity on appropriate substrates in the presence of univalent and bivalent antibodies.

Major Findings: During the past two years, the investigator has demonstrated that rabbit spermatozoa treated with univalent anti-rabbit semen antibody fail to fertilize ova when placed into the vagina or the uterus. In the new experiments antibody treated capacitated sperm were placed into oviducts and the fertilization rate of ova was ascertained. Treatment of rabbit sperm with guinea pig anti-rabbit antibody blocked fertilization. Utilization of goat antibodies produced ambiguous results in that both immune and control globulins prevented fertilization. This was not true with the vaginal and uterine inseminations of uncapacitated sperm. From this standpoint, it is felt that the control goat globulin may prove to be an important marker for an essential "fertilization substance" accessible only on capacitated sperm.

Numerous experiments concerned with the species specificity of the anti-sperm (hyaluronidase) antibody indicated surprising specificity. Thus, univalent goat anti-rabbit semen antibodies inhibit hyaluronidase activity and corona dispersing activity (CDA) of rabbit epididymal ejaculated and capacitated sperm but not bovine testicular hyaluronidase or guinea pig, mouse or rat CDA. Also, rabbit antihuman semen globulin inhibits human sperm hyaluronidase activity, but not rabbit or bovine sperm hyaluronidase.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies which utilize immunological techniques to study reproductive processes.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Neurochemical Control of Sperm Motility
Contractor: Medical College of Ohio
Money Allocated: $45,558 (FY 70), $45,558 (FY 71), $48,330 (FY 72)

Objectives: The investigator will apply biochemical, electron microscopic, and electrophysiological procedures to the study of control of flagellar movement as related to sperm motion. More specifically, the applicant will conduct a systematic analysis of the role of acetycholinesterase in the regulation of the propulsive motion of spermatozoa. The study will be conducted in three phases:

1. Sperm cell suspensions will be subjected to microcolorimetric analysis of acetycholinesterase activity, rating of motility, timing of swimming duration, electron microscopic cytochemical procedures, and microelectrode determinations of electrical potentials. The effects of specific acetycholinesterase inhibitors, such as eserine, on sperm propulsion will also be studied.

2. In the second part, the investigator will examine the developmental and maturational aspects of flagellar growth in relation to the development of sperm motility.

3. The third part will be directed toward the environmental interactions, both physical and chemical, between the sperm cells and the medium.

Major Findings: During the second year, the investigator has studied the electron microscopy distribution of acetylcholinesterase in spermatids and immature spermatozoa. Because of differences in cytochemical reactivity depending on the substrate and on the type of acetylcholinesterase activity used, it is concluded that two cholinesterasic enzymes coexist. During differentiation they reassemble from a generalized cytoplasmic particulate distribution in the early spermatid to a concentration in the neck region as the flagellum elongates. The residual body and the cytoplasmic droplet retained a substantial enzymic activity. As the spermatid elongation progress, the enzymes become associated with the mitochondria membranes and the fibers of the flagellum. Continued studies on invertebrate spermatozoa indicate that dimethylsulfoxide inhibits motility in a dose-dependent relationship. Acetylcholin chloride added to the system doubled the swimming rate and hemicholinium which blocks choline transport through biological membranes, decreases sperm motility markedly. These experiments confirm the involvement of acetylcholinesterase system in the regulation of sperm motility. Electrophysiological studies have continued. The emphasis has been on the influence of the ionic composition of the medium on the bioelectrical behavior of the sperm. Potassium appears to be the most indispensable ion. Its depletion from the medium immobilize the cells and raises the electric potential. Its excess is equally deleterious; the recorded potential drops and the motility changes to a low amplitude twitch pattern. Sodium is necessary but the sperm respond with considerable less sensitivity to alteration in sodium concentration. Chloride

may be completely replaced by methylsulfate for example. Calcium ions are quite deleterious to the bioelectrical potential and motility.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on sperm motility.

Proposed Course: This is a continuing contractual effort and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Neurochemical Control of Sperm Motility
Contractor: Medical College of Ohio
Money Allocated: $45,558 (FY 70), $45,558 (FY 71), $48,330 (FY 72)

Objectives: The investigator will apply biochemical, electron microscopic, and electrophysiological procedures to the study of control of flagellar movement as related to sperm motion. More specifically, the applicant will conduct a systematic analysis of the role of acetycholinesterase in the regulation of the propulsive motion of spermatozoa. The study will be conducted in three phases:

1. Sperm cell suspensions will be subjected to microcolorimetric analysis of acetycholinesterase activity, rating of motility, timing of swimming duration, electron microscopic cytochemical procedures, and microelectrode determinations of electrical potentials. The effects of specific acetycholinesterase inhibitors, such as eserine, on sperm propulsion will also be studied.

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3. The third part will be directed toward the environmental interactions, both physical and chemical, between the sperm cells and the medium.

Major Findings: During the second year, the investigator has studied the electron microscopy distribution of acetylcholinesterase in spermatids and immature spermatozoa. Because of differences in cytochemical reactivity depending on the substrate and on the type of acetylcholinesterase activity used, it is concluded that two cholinesterasic enzymes coexist. During differentiation they reassemble from a generalized cytoplasmic particulate distribution in the early spermatid to a concentration in the neck region as the flagellum elongates. The residual body and the cytoplasmic droplet retained a substantial enzymic activity. As the spermatid elongation progress, the enzymes become associated with the mitochondria membranes and the fibers of the flagellum. Continued studies on invertebrate spermatozoa indicate that dimethylsulfoxide inhibits motility in a dose-dependent relationship. Acetylcholin chloride added to the system doubled the swimming rate and hemicholinium which blocks choline transport through biological membranes, decreases sperm motility markedly. These experiments confirm the involvement of acetylcholinesterase system in the regulation of sperm motility. Electrophysiological studies have continued. The emphasis has been on the influence of the ionic composition of the medium on the bioelectrical behavior of the sperm. Potassium appears to be the most indispensable ion. Its depletion from the medium immobilize the cells and raises the electric potential. Its excess is equally deleterious; the recorded potential drops and the motility changes to a low amplitude twitch pattern. Sodium is necessary but the sperm respond with considerable less sensitivity to alteration in sodium concentration. Chloride

may be completely replaced by methylsulfate for example. Calcium ions are quite deleterious to the bioelectrical potential and motility.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on sperm motility.

Proposed Course: This is a continuing contractual effort and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Protease Inhibitors in Human Genital Secretions
Contractor: The University of Chicago
Money Allocated: $23,227 (FY 70), $11,614 (FY 71), $19,282 (FY 72)

Objectives: The investigator will study the possibility of a protease-inhibitor relationship of biological significance in the interaction between seminal fluid and sperm on the one hand and female genital tract secretions on the other. Alpha-1-antitrypsin has been discovered in cervical mucus and its level is governed by sex hormones. Other inhibitors may also be present in seminal fluids and in the female tract.

Major Findings: Three protease inhibitors have been found in human cervical secretions, in addition to α_1-antitrypsin (α_1AT): inter-α-trypsin inhibitor (I_αTI), α_{1_X}-antichymotrypsin (α_{1_X}ACHY) and antithrombin III (AT III). α_1AT, I_αTI and α_{1_X}ACHY have been quantitatively determined during presumably ovulatory cycles and under administration of sequential contraceptive hormones. The level of these inhibitors is apparently influenced by sex hormones. The inhibitors are present in extremely low concentrations or absent in preovulatory cervical mucus or in mucus specimens obtained while estrogens were administered. This is the type of mucus that is favorable for sperm penetration. A significant increase has been observed shortly after ovulation or during administration of gestagens. Sperm cannot penetrate into mucus of this type. Human seminal fluid shows very low concentrations of α_1-antitrypsin and α_{1_X}-antichymotrypsin and only traces or absence of inter-α-trypsin inhibitor. A low molecular weight seminal plasma trypsin inhibitor was partially purified. Acrosomal extracts were prepared from rabbit epididymal and from human ejaculated spermatozoa. Proteolytic activity and inhibitor effects were tested on BAEE and gelatin as substrate. Purified human α_1-antitrypsin of the immediate type inhibits human and rabbit acrosomal protease progressively, inter-α-trypsin inhibitor inhibits the enzyme immediately and α_{1_X}-antichymotrypsin does not inhibit acrosomal protease. The trypsin-kallikrein inhibitor from bovine lung, a trypsin inhibitor of the immediate type, inhibits rabbit and human acrosomal protease progressively. The progressive type of kinetics of α_1-antitrypsin and trypsin-kallikrein inhibitor of bovine lung indicates significant differences between acrosomal protease and trypsin.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support research on sperm inhibitors.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Testis and Sperm Specific Antigens of Man and Certain Other Primates
Contractor: University of Michigan
Money Allocated: $21,140 (FY 71), $21,768 (FY 72)

Objectives: Immunization with testis and sperm antigens is a possible means of human contraception. However, the use of the materials will depend upon knowledge of the characterization and purification of these antigens. This proposal is intended to yield information on the chemical and immunologic nature of immunogens specific for testis and sperm, the possible influence of the epididymis on the antigenic molecules, and the effects of antibodies against these antigens on sperm viability and function. The use of immunesera from infertility patients will permit detection of those testes and sperm antigens that are immunogenic in man and their possible relationship to infertility. These immunogens would be purified and characterized. The effects of antibodies induced by the different antigens upon sperm will be determined by laboratory tests involving agglutination, immobilization and cervical mucus penetration. Possible changes in the chemistry of sperm antigens in the epididymis will be associated with the maturation of sperm cells. After characterization and purification of the immunogens future plans call for isoimmunization of male and female monkeys to determine the effects on the fertility process.

Major Findings: Rabbit antisera against human testis homogenate exhibited sperm agglutinating and immobilization activities, as well as precipitation reactions against testis extract in immunodiffusion and electrophoresis. Absorption of the human semen and spleen cells to remove non-specific antibodies resulted in a loss of activity in the majority of the antisera. Production of new antisera in rabbits after a partial induction of tolerance to serum and spleen antigens has given better specificity and strength of antibodies. Three to five specific testis antigens have been detected in precipitation reactions by the different antisera. Antisera against both rhesus and baboon testes produced, even after absorption with serum and spleen, rapid immobilization of human sperm and one precipitin line with human testis in gel diffusion.

Extraction and solubilization of the antigens of human testis with citric acid and Triton X-100, followed by column chromatography using Sephadex G-200 has resulted in four major peaks. The specific antigen inducing sperm immobilization was detected in the first peak by use of immune sera from two vasectomy patients. The immobilizing antigen is associated with or is the antigen that has alpha globulin mobility in immunoelectrophoresis.

Significance to Biomedical Research and Program of the Institute: Immunologically induced infertility could be a useful contraceptive method and its study

is highly relevant to the contraceptive development program especially since the work will be done on primate material.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Electron Spin Resonance and Radioisotope Labelling Studies of Mammalian Reproductive Cell Membranes in the Evaluation of New Methods of Contraception
Contractor: University of Hawaii School of Medicine
Money Allocated: $56,099 (FY 70), $66,606 (FY 71), $68,387 (FY 72)

Objectives: In this research project, electron spin resonance (ESR) spin-labelling, will be applied to the study of sperm membrane interactions and sperm transport in vivo. The method relies on the ease of labelling sperm cells with a lipid-seeking spin-label which causes no loss of sperm motility or function. The spin-label is capable, through analysis, of reflecting structural changes in the membrane of the sperm. These changes will be measured during sperm capacitation and interaction of sperm with genital fluids and chemical contraceptives. The same kind of label, but tagged with C^{14}, will be used to measure in vivo transport of sperm in the uterine tract of experimental animals especially primates.

Following preliminary work aimed at establishing the feasibility of using such a spin-label for detecting alterations in sperm membrane characteristics, such studies would be extended to the in vivo case. Miniaturized electron spin resonance detectors would be developed for implantation directly in the tract so that alterations could be monitored directly. The investigators also propose to use a radioisotope label for studying sperm transport within the female tract. In this case the sperm would be labelled with a fatty acid carrying not a paramagnetic nitroxide, but a heavy concentration of C^{14}; the investigators have designed a small Geiger-Mueller tube (2mm diameter, 5mm in length) which could be implanted in the uterine tract to detect in vivo transport of the labelled sperm.

Major Findings: The investigators have had partial success in finding spin labels that bind to the membranes of living gametes anddo not disrupt capacitation. With the spin labels currently in use, concentrations low enough to ensure that capacitation is not impaired are inconsistent with the signal-to-noise ratio measurements for close interpretation of data. The high sensitivity cavity, that should arrive shortly, will enhance the interpretation of experiments that involve the membrane modifications associated with capacitation. However, at present state of art, the amount of label that can be attached to sperm without either killing them or preventing them from capacitating is barely within the sensitivity range of ESR apparatus and they are having difficulty seeing whether there are any spectral changes due to capacitation.

There are significant differences in the ESR spectra of sperm from different regions of the epididymis. It is clear that there is a substantial increase in the amount of hindrance of the spin label as the sperm transverse the epididymis of the rabbit and rat. This supports the findings of Fawcett and

Phillips (1969) and Bedford and Nicander (1971) that changes occur in the acrosome and in the membranes of sperm during their passage through the epididymis.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly related to the published purpose of the Contraceptive Development Branch to support studies which make use of state-of-the-art technology to study reproductive processes such as sperm capacitation and transport.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Contraceptive Development Studies for Males: Oral Steroid Hormone Administration
Contractor: University of Washington School of Medicine
Money Allocated: $118,056 (FY 71) for two years

Objectives: This contract proposal is designed to evaluate the combined effects of two androgenic steroids on spermatogenesis when administered to normal adult human males. The ultimate goal is to develop an effective oral contraceptive technique for males.

At the present time there is no compound available for effectively inhibiting human spermatogenesis which is non-toxic or absent from adverse reactions which would render the preparation either unacceptable to the patient or to his physician.

Major Findings: Due to difficulties in obtaining a suitable patient population this contract is in its initial phase.

Significance to Biomedical Research and Program of the Institute: Development of contraceptive techniques for the male is highly relevant to the contraceptive development program.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Hormonal Control of the Epididymis
Contractor: Bureau of Biological Research, Rutgers University
Money Allocated: $62,539 (FY 72) for two years

Objectives: The primary objective of this research is to determine the biochemical composition of rat epididymi under a variety of hormonal conditions. Among the biochemical endpoints that will be evaluated are: (1) total protein, (2) nucleic acids, (3) alanine and aspartic transaminases, (4) glycogen, (5) glucose-6-phosphate and 6-phosphogluconate dehydrogenases, (6) free amino acids, (7) fatty acids, (8) lactic dehydrogluase and alkaline phosphatase.

The influence of the following hormones and hormone antagonists will be evaluated: (1) various androgens, (2) estradiol, (3) cortisol and (4) cyproterone acetate, A-nor progesterone and MER-25.

The influence of castration or the deletion of testicular fluid (efferentiectomy) on epididymal composition will be assessed.

The biochemical assessment will be carried out on the cauda and the caput of young adult male rats.

Significance to Biomedical Research and Program of the Institute: The study of epididymal function is one of the stated goals of the contraceptive development program.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Ultrastructural Changes in the Sertoli Cells in Rats Under Various Hormonal Conditions
Contractor: University of Texas Medical School, Houston
Money Allocated: $126,804 (FY 72) for three years

Objectives: The objective and scope of the present study is to conduct a detailed investigation of the ultrastructure of mature and developing Sertoli cells of rats under various experimental conditions. Specific emphasis will be placed on the possible hormonal interaction which may be operative in Sertoli cells. In vivo and in vitro studies will be made of testes from developing and adult animals deprived of gonadotropic hormones, replaced with gonadotropins or androgens or treated with a cytotoxic agent, furadroxyl.

Significance to Biomedical Research and Program of the Institute: Understanding of the regulatory function of the Sertoli cells may suggest approaches to interference with seminiferous tubule function. The study is clearly relevant to the goals of the program.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Use of Reversible Vasectomy Device in the Guinea Pig
Contractor: New York Medical College
Money Allocated: $70,000 (FY 71), $59,933 (FY 72)

Objectives: The purpose of this project is to ascertain the usefulness of a reversible intravasal valve as a contraceptive device in the male guinea pig. Because of the confidential nature of this aspect of the CDB program, disclosure of the nature of the device cannot be made until release of information will be allowed.

Major Findings: The investigator has implanted the Bionyx Control (reversible vasectomy device) in 10 guinea pigs in the "on" position and in 10 animals in the "off" position. Neither blockage in all animals bearing the device in the "off" position nor unimpeded passage of sperm in all animals bearing the device in the "on" position was achieved . Results are encouraging, however, and failures appear related to the operative procedure and subsequent tissue growth. Adverse tissue reaction and corrosion of the valve have not been observed.

Significance to Biomedical Research and Program of the Institute: It would be important to determine the usefulness of this contraceptive device as the development of devices for male sterilization is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Research and Development of Contraceptive Devices and Materials. Development of New Sterilization Techniques
Contractor: Abcor, Inc., Cambridge, Massachusetts
Money Allocated: $345,000 (FY 71); for two years

Objectives: The purpose of this project is to develop (1) a reversible intravasal plug with a hollow center into which a removable pin can be inserted, and (2) an absorbable intravasal stent that would facilitate reanastomosis of the cut end of the vas following vasectomy. A variety of materials will be evaluated for the devices and the effectiveness of these new sterilization techniques will be studied in the guinea pig.

Major Findings: The contractor has prepared a number of reversible vasectomy devices and intravasal stents that are presently undergoing animal experimentation.

Significance to Biomedical Research and Program of the Institute: It would be important to determine the utility of these contraceptive devices as the development of devices for male sterilization is directly relevant to the purposes of the Contraceptive Development Branch activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Design and Development of Implantable Contraceptive Devices for Use in the Male
Contractor: IIT Research Institute
Money Allocated: $150,000 (FY 71) $194,476 (FY 72)

Objectives: The purpose of this project is to conduct a feasibility study on the design of devices suitable for intravasal implantation for the purpose of reversibly occluding the vas. Biological studies will be conducted in animals to determine the reversibility and tissue compatibility of the devices.

Major Findings: These investigators have undertaken a broad interdisciplinary approach to the study of devices designed for vasal occlusion. Progress has been made in the morphological comparison of dog and human vasa including SEM and gauging studies. Luminal diameter gauges and reamers (to remove the mucosa) have been fabricated and tested. Stainless steel alone and with a ceramic coating have been implanted in the vas to determine tissue reaction. Several prototype valves have been designed and fabricated and two of these devices have been implanted in dogs. A number of biomaterials are under study to determine their potential usefulness in the fabrication of occlusive devices especially in regard to the surface characteristics essential for proper tissue ingrowth.

Significance to Biomedical Research and Program of the Institute: The design and development of implantable devices for male sterilization is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Feasibility Study of Intrauterine Microcapsules as a Prolonged Drug Release Form
Contractor: Battelle Memorial Institute
Money Allocated: $61,200 (FY 71), $69,980 (FY 72)

Objectives: The purpose of this project is to investigate the feasibility of employing a microencapsulated drug dose form as an intrauterine antifertility measure. Feasibility will center largely on maintaining microcapsules within the uterus. The drug delivery system would involve the microencapsulation of progestagens, spermatocidal agents, or cervical mucosal thickeners. After microencapsulation of the designed drug agent, these microcapsules would be inserted in the uterus for a controlled and prolonged release of the drug.

Major Findings: The investigators have successfully encapsulated a variety of core materials using cellulose acetate butyrate as the wall membrane. The core materials have been either an aqueous phase or an oil emulsion. The encapsulation of the silicone-in-water emulsion is a significant breakthrough, because it permits encapsulation of both hydrophobic and hydrophilic materials by the non-aqueous phase separation method. Various capsule sizes ranging from 50μ to 1600μ have been prepared.

Encapsulation of both "cold and hot" progesterone has been successful. Release rates of progesterone in vitro into buffered media have been calculated. Release into phosphate buffers was increased when a surfactant was added to the buffer.

Studies on capsule behavior in rabbit reproductive tracts have been initiated. Capsules of the size 840μ and 1680μ stay within the uterus for at least 7 days. They retain their integrity and do not produce pathological changes in the endometrium. Examination of rabbits which had uterine instillation of the 740μ and 1680μ capsules thirty-six days after instillation has indicated absence of capsules in the uterine cavity. Capsules were found in the peritoneal cavity and surprisingly these large size capsules were found in the oviducts. It is not known at the present time whether the oviductal location of the microcapsules is the result of migration from the uterus or is due to their pickup from accidental spillage into the peritoneal cavity.

Significance to Biomedical Research and Program of the Institute: It would be important to conduct a feasibility study of intrauterine microcapsules as a prolonged drug release form as the development of intrauterine antifertility measures is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Grafted Hydrogels in Contraceptive Application
Contractor: Franklin Institute Research Laboratories
Money Allocated: $108,356 (FY 71), $3,035 (FY 72)

Objectives: The purpose of this project is to prepare hydrogel-coated intra-uterine devices and hydrogel-coated intra-uterine devices impregnated with copper and to study these devices in rabbits to determine their tolerance and effectiveness as antifertility devices. Conventional IUD's have already shown promise and it is anticipated that there will be greater tolerance and less medical problems with a hydrogel-coated IUD than with the conventional IUD. A series of studies will be performed with complete, scaled devices on the rabbit initially and then based on these animal results, in the human.

Major Findings: The contractor has prepared hydrogel coated polyethylene IUD's. Initial studies in the rabbit indicate better retention of the new IUD's in the uterine cavity.

Significance to Biomedical Research and Program of the Institute: The development of hydrogel-coated IUD's for female sterilization is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1970 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development of an Intra-uterine Sterilization System for the Female
Contractor: IIT Research Institute
Medical Engineering Center
Money Allocated: $247,977 (FY 71), $345,246 (FY 72)

Objectives: The purpose of this project is to develop a prototype system for intra-uterally sterilizing the female by occlusion of the utero-tubal junction. The occlusive devices would be introduced through the cervical os, and be guided into the tubal location by a specially designed steerable hysteroscope. This would simplify the procedure for the physician or specially trained paramedical personnel, in a non-operative procedure. The design and development effort will be supported with biological and animal experiments and will lead eventually to clinical use in humans.

Major Findings: The contractor has made excellent progress in the development of a prototype steerable hysteroscope. Using a unique cervical seal to ensure minimal leakage of the distending fluid, good photographs of the tubal openings have been obtained in excised uteri shortly after hysterectomy. Plugs have been placed within the intramural portion of the oviduct under direct observation and their location confirmed by dissection.

Several occlusive devices have been designed and fabricated using porous and ceramic coated stainless steel, ceramic containing a heating element, and silicone rubber with and without etched stainless steel rings to stimulate tissue ingrowth. Surgical insertion of stainless steel plugs in the rabbit showed fibrous tissue ingrowth.

Significance to Biomedical Research and Program of the Institute: The development of a system for intra-uteral sterilization of the female is directly relevant to the purposes of the CDB activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: A Proposal for the Study of a Long Acting Injectable Progesterone Contraceptive
Contractor: Brookdale Hospital Center
Money Allocated: $116,512 (FY 71); for two years

Objectives: The purpose of this project is to prepare and evaluate two progesterone contraceptive drug release systems to determine the possibility of designing a progesterone preparation which would release relatively constant amounts of the agent sufficient for contraceptive purposes for at least three months. The two systems involve steroid-lipid pellets and collagen implants. Steroid lipid pellets will be prepared by processing eutectic mixtures of progesterone with various lipids, primarily sterols, in an apparatus permitting preparation of pellets of any desired diameter. Collagen implants will be made by binding progesterone to collagen solution and inducing varying degrees of crosslinking. In vitro and in vivo studies in hamsters and rabbits will be undertaken to determine the rate of dissolution of these different matrices and the rate at which progesterone will be liberated.

Major Findings: The contractor has prepared a variety of eutectic mixtures of progesterone with various lipids. The dissolution rates in vitro are presently being investigated.

Significance to Biomedical Research and Program of the Institute: The development of progesterone drug release systems for female contraceptive measures is directly relevant to the purposes of the Contraceptive Development Branch activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Device and Technique for Blocking Fallopian Tubes
Contractor: Franklin Institute Research Laboratories
Colloids and Polymers Laboratory
Money Allocated: $78,866 (FY 71)

Objectives: The purpose of this project is to develop a new device and application technique for accomplishing the blocking of fallopian tubes with medical-grade silicone rubber for reversible sterilization. The device is to be evaluated in rabbits for installation, compatibility, antifertility efficacy and reversibility.

Major Findings: A non-air-entraining mixer-dispenser and silicon-rubber obturator which was developed made it possible to produce successful installations in rabbits. Thirty-day retention of properly installed oviduct-blocks was demonstrated. Also demonstrated were removal of the blocking structure in one piece from the uterine side (for reversibility) and freedom from adverse tissue reactions. Rabbit studies for antifertility efficacy and further development of prototype devices for humans are to be carried out in the balance of the contract period.

Significance to Biomedical Research and Program of the Institute: The development of a device for accomplishing the blocking of fallopian tubes for female sterilization is directly relevant to the purposes of the Contraceptive Development Branch activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: New Hydrophilic Polymers for Population Regulation
Contractor: Polysciences, Inc.
Polymer Laboratories
Warrington, Pennsylvania
Money Allocated: $74,419 (FY 71)

Objectives: The purpose of this project is to develop new hydrophilic polymers a) for induction of abortion through cervical dilation and b) for extending the life of vaginal spermicidal agents. For induction of abortion using hydrophilic polymers a study of various biologically inert materials will be made in vitro and an experimental study will be conducted in animals. This will be followed by a very limited clinical trial. The study of a substantive hydrophilic vaginal spermatocidal contraceptive will be limited to animals and will ascertain the duration of action of spermatocidal activity as compared to available creams and foams.

Major Findings: The contractor has prepared dehydrated rods manufactured from hydrophilic polymer gels. Insertion of such rods into the rabbit cervix results in dilation of the cervix. In pregnant rabbits abortion could not be induced through cervical dilation accomplished with present gels.

Significance to Biomedical Research and Program of the Institute: The development of hydrophilic polymers for induction of abortion and for extending the life of spermicidal agents as a female contraceptive device is directly relevant to the purposes of the CDB activities.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1972 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Reversible Sterilization by an Intravasal Spermicidal Device
Contractor: Southwest Foundation for Research and Education
Money Allocated: $41,678 (FY 72)

Objectives: The objective of this research is to develop an intravasal device which is spermicidal in its effect. This device is intended to produce reversible sterilization of the male without blocking sperm passage through the male tract. Such an approach may eliminate the potential immunological side effects of those devices which produce sterilization by temporary or permanent blockage of the vas deferens.

Significance to Biomedical Research and Program of the Institute: Development of new techniques and devices for production of reversible sterilization is one of the stated goals of the program.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new contraceptive devices and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: A Study of the Factors that Control Diffusion Rates in Controlled-Release Contraceptive Systems.
Contractor: Southern Research Institute
Money Allocated: $64,354 (FY 72)

Objectives: This proposal aims to quantify individual factors affecting diffusion rate of steroidal contraceptive drugs through polymeric materials (membranes). Diffusion rate is the combined effect of the solubility of the drug in the polymer, the diffusivity of the drug in the polymer and the partition coefficient between the drug in the polymer and the solution into which it is diffusing.

The study will show the difference in the transport rates between the various drugs and polymers, the influence of the above physical constants on the rate and also indicate whether dissolution or diffusion of the drug in the polymer is rate limiting. Further, an attempt will be made to establish the relationship between the drug structure and the structure of the polymer in the diffusion process.

Significance to Biomedical Research and Program of the Institute: The study will provide much needed information on the type of polymer to be used with a given steroid to either inhibit or enhance the rate of drug release and is therefore directly relevant to the objectives of the CDB.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Development and Evaluation of a Reversible Vasectomy Prosthesis
Contractor: University of Missouri
Money Allocated: $38,290 (FY 72)

Objectives: This research is directed toward the development of a reversible vasectomy prosthesis as well as parallel studies of the effects of the prosthesis and of temporary vas occlusion on gametogenesis.

Particular attention will be paid to the effect of the prosthesis on the tissues of the vas deferens over a prolonged period of time. Tissues will be evaluated by histopathologic studies.

The effects of temporary occlusion of the vas on gametogenesis will be evaluated following semen collection and evaluations. The testes will later be studied histologically and gametogenesis further evaluated.

In order to accomplish these studies a vasectomy prosthesis of silastic has been designed. This prosthesis is inserted into the lumen of the vas and the musculature of the vas sutured to a cuff on the prosthesis. Occlusion is obtained by folding the tube on itself and ligating it. Sperm passage is obtained by removing the ligature and allowing the tube to become patent.

The initial studies will focus on development of the tube design, surgical implantation of the tube, effects on the vas deferens for short periods, and the effects of occlusion for 5-6 month periods on gametogenesis.

Later studies will evaluate the effects of the tube on the vas deferens and gametogenesis for longer periods of time.

Significance to Biomedical Research and Program of the Institute: Study of reversible vasectomy devices and of their effects on reproductive function is the goal of the program aimed toward the development of new contraceptive technology.

Proposed Course: This is expected to be a continuing contractual effort leading to the development of new contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis of Prostaglandin Analogs for Biological Studies
Contractor: University of Chicago
Money Allocated: $46,675 (FY 71), $20,943 (FY 72), $65,397 (FY 73)

Objectives: The purpose of this project is to prepare prostaglandin-like compounds in chemically pure form and in sufficient quantities for evaluation of their effects on fertility. Another objective is to develop methods for the large-scale synthesis of $PGF_{1\alpha}$ and $PGF_{2\alpha}$ in both the natural form and as their optical antipodes and 15 epimers.

Major Findings: Sixteen prostaglandin analogs have been synthesized and methods have been developed for the large-scale synthesis of $PGF_{2\alpha}$. Major biological findings are not available at this early stage of development.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandin-like compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis and Antifertility Testing of Prostanoic Acid Derivatives
Contractor: Ayerst Laboratories
Money Allocated: $180,640 (FY 71); for two years

Objectives: The purpose of this project is to synthesize a series of prostanoic acid derivatives and to test their antifertility effect in animals.

Major Findings: This contract involves the handling of large quantities of chemical intermediates in multistep syntheses in order to obtain a sufficient quantity of chemically pure material for biological evaluation. Due to the synthetic complexities involved, this contract has not significantly advanced beyond some preliminary biological evaluation of several of the required compounds. Several of the prostanoic acid derivatives were found to be effective fertility regulating agents in hamsters at higher doses than $PGF_{2\alpha}$.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandin-like compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesize Nitrogen-Containing Analogs of Prostaglandins
Contractor: University of New Hampshire
Money Allocated: $27,418 (FY 72)

Objectives: The purpose of this project is to synthesize azaprostaglandin analogs and to test these compounds for antifertility activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandin analogs for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Structure-Activity Studies of Small Peptides which Release LH and FSH.
Contractor: Tulane University
Money Allocated: $59,171 (FY 72)

Objectives: The purpose of this project is to biologically evaluate new synthetic polypeptides, which may affect the reproductive system, based upon structural modifications of (1) the decapeptide, LH-RH/FSH-RH and (2) a synthetic tetrapeptide which was found to release LH, but not FSH.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of LH-RH/FSH-RH analogs for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesize and Furnish Peptide Inhibitors of Luteinizing Hormone-Releasing Factor/Follicle Stimulating Hormone-Releasing Factor
Contractor: University of Wisconsin
Money Allocated: $35,560 (FY 72)

Objectives: The purpose of this project is to synthesize competitive inhibitors of LH-RH/FSH-RH by replacing certain hydrophobic amino acid residues in LH-RH/FSH-RH by unusual amino acids to increase the hydrophobic properties of the amino acid normally present in that position while providing a minimal steric hindrance between the releasing hormone at that position and its receptor. Such competitive inhibitors are potential antifertility agents.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of LH-RH/FSH-RH analogs for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis and Testing of Gonadotropin Releasing Hormone (Gn-RH) to Establish Antifertility and Other Endocrine Activity
Contractor: Abbott Laboratories
Money Allocated: $125,300 (FY 72)

Objectives: The purpose of the project is to (1) develop methods for the large-scale synthesis of the gonadotropin releasing hormone, (2) develop and evaluate chemical and biological tests to establish the integrity of the product and the reproducibility of different batches, (3) subject the synthetic hormone to physiological, pharmacological and toxicological testing in laboratory animals, (4) make at least ten grams of the synthetic hormone available to the CDB, (5) prepare a brochure of information on the hormone for use by clinical investigators, and (6) apply for an IND.

Significance to Biomedical Research and Program of the Institute: The work undertaken to synthesize Gn-RH and conduct extensive biological studies in laboratory animals to establish antifertility and other endocrine activity is an integral part of the contraceptive development program.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title : Production of New Type of Contraceptives Based on Derivatives of Luteinizing Hormone-Releasing Hormone (LH-RH).
Contractor: Tulane University
Money Allocated: $51,716 (FY 72)

Objectives: The purpose of this project is to prepare and evaluate biologically decapeptide analogs of LH-RH which involve modifications of the histidine residue, the serine residue and the tyrosine residue in order to produce an inhibitor of LH-RH.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of LH-RH analogs for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis and Testing of Antifertility Compounds
Contractor: Stanford Research Institute
Money Allocated: $108,908 (FY 71), $82,798 (FY 72)

Objectives: The purpose of this project is to synthesize a series of heterocyclic compounds and silicon-containing steroidal compounds and test their antifertility effect in rats.

Major Findings: The introduction of silicon into certain steroids markedly affects their biological response and causes a favorable separation of antifertility activity from estrogenic activity. In postcoital assays in rats, one of the silicon-containing steroids was found to be at least four times as active as ethynyl estradiol (EE) as an antifertility agent, but shows only 75% of the estrogenic potency of EE. Further exploration of this phenomenon into androgenic and progestagenic compounds is in progress.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of steroidal and non-steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

Contract Title: Synthesis of Prostaglandin Analogs
Contractor: Research Triangle Institute
Money Allocated: $51,089 (FY 72)

Objectives: The purpose of this project is to synthesize 10-oxa-prostaglandin analogs lacking the 11-oxygen function and to test these compounds for antifertility activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of prostaglandin-like compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

Contract Title: Synthesis of Novel Compounds of Potential Anti-Fertility Activity
Contractor: University of California
Money Allocated: $23,994 (FY 72)

Objectives: The purpose of this project is to synthesize cis- and trans-4,6-cyclopropane analogs of certain contraceptive steroids which, due to the unique geometrical conformations imposed by the introduction of the cyclopropane ring, could competitively inhibit progesterone at the receptor site and result in an antiprogestational compound. These compounds will be tested for antifertility activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesize Novel Derivatives of Progesterone and Testosterone
Contractor: University of Pittsburgh
Money Allocated: $66,779 (FY 72)

Objectives: The purpose of this project is to prepare unique chemical modifications of certain contraceptive steroids which, due to the introduction of unsaturation and the 10α (retrograde) configuration, will produce changes in the geometry of the steroidal conformation and thus might lead to selective changes in steroidal receptor attachments and therefore to interesting physiological consequences. These steroids will be tested for antifertility activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Synthesis of Potential New Agents for the Control of Fertility
Contractor: University of Rhode Island
Money Allocated: $26,660 (FY 72)

Objectives: The purpose of this project is to prepare 9(10→19) abeo-steroids (substances related to 19-nor steroids but having an extra methylene group be between ring A and C) and to test their antifertility and hormonal effects in rats.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Synthesis and Testing of Silicon Substituted Steroids as Potential Antifertility Compounds
Contractor: Research Triangle Institute
Money Allocated: $30,312 (FY 72)

Objectives: The purpose of this project is to synthesize silicon substituted steroids related to estradiol and to test these steroids for antifertility and estrogenic activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesize and Furnish Steroidal Compounds with Antifertility Activity
Contractor: Laval University
Money Allocated: $110,772 (FY 72); for two years

Objectives: The purpose of this project is to synthesize 11-oxa analogs of certain contraceptive drugs for biological evaluation of their effects on the reproductive system. Preliminary biological evaluation indicates that 11-oxaprogesterone has enhanced ovulation inhibiting activity and reduced progestational activity on subcutaneous administration when compared with progesterone.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of steroidal compounds for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Synthesizing, Furnishing and Testing of Antifertility Compounds
Contractor: Research Triangle Institute
Money Allocated: $112,855 (FY 71)

Objectives: The purpose of this project is to synthesize a series of steroidal and non-steroidal compounds and to test their antifertility effect in mice.

Major Findings: Several metabolites of norethindrone and norethynodrel have been synthesized and evaluated for biological activity. None of the metabolites were found to display any favorable biological properties relative to the parent contraceptive compounds.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of steroidal and non-steroidal compounds for contraceptive utility.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Investigation of the Antifertility Activity in the Monkey and Long-Term Biological Effects in the Rat, Dog and Monkey of a Novel, Synthetic Steroid
Contractor: Lederle Laboratories Division, American Cyanamid Company
Money Allocated: $65,749 (FY 71)

Objectives: The purpose of this project is to synthesize a novel steroidal compound (CL 61066) and to test its antifertility effect in rats, rabbits, and monkeys.

Major Findings: This novel progestational compound showed high antifertility activity in the rat and the rabbit. The anticipated antifertility activity in the monkey was never realized and even at dose levels of 500 µg/kg/day, 3/5 monkeys become pregnant.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of steroidal compounds for contraceptive utility.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: The Provision and Synthesis of Certain Alkylating Steroids and the Testing of Their Possible Antifertility Effects
Contractor: Southern Illinois University
Money Allocated: $29,357 (FY 71)

Objectives: It is the purpose of this project to synthesize and test three steroidal compounds for antifertility activity in mice.

Major Findings: One of the three compounds evaluated for biological activity has exhibited modest antifertility activity but it appears to be directly related to its estrogenic activity.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purpose of the Contraceptive Development Branch to support the synthesis and evaluation of steroidal compounds for contraceptive utility.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis, Characterization, and Biological Testing of Specifically Designed Anti-implantational Agents
Contractor: Boston University
Money Allocated: $67,500 (FY 71)

Objectives: The purpose of this project is to test the hypothesis that compounds exhibiting activity as competitive antagonists of estradiol in uterine tissue are useful as antifertility agents because they may be devoid of estrogenic activity or have a large separation of antifertility and estrogenic activity. A series of steroidal compounds, synthesized or obtained from other sources, are to be tested as competitive inhibitors of estradiol against the 8S and 5S receptors and then tested for antifertility activity in rats, rabbits, and hamsters.

Major Findings: A large number of compounds were tested for ability to compete with ^{3}H-estradiol for binding in the nuclear and non-nuclear fraction of rat uteri 2 hours after s.c. injection. A large number of estrogen analogues were tested for anti-implantation and estrogenic (i.e. uterotrophic) activity. Of these, 16-epiestriol exhibited the highest anti-implantation to estrogenic ratio. The effect of 16-epiestriol on estradiol binding was in the intermediate range. Estriol was also found to interfere with the binding of estradiol.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of steroidal compounds for contraceptive utility.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesizing and Testing of Antifertility Compounds
Contractor: Collaborative Research, Inc.
Money Allocated: $137,316 (FY 71) for two years

Objectives: It is the purpose of this project to synthesize a series of polyheterocyclic compounds and to test their antifertility effect in mice and rats.

Major Findings: The synthesis of some of the polyheterocyclic compounds has been completed. Major biological findings are not available at this early stage of development.

Significance to Biomedical Research and Program of the Institute: The work undertaken to synthesize these polyheterocyclic compounds and test them for antifertility activity is directly relevant to the purposes of the Contraceptive Development Branch activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesis, Characterization, and Biological Testing of Specifically Designed Anti-implantational Agents
Contractor: Boston University
Money Allocated: $67,500 (FY 71)

Objectives: The purpose of this project is to test the hypothesis that compounds exhibiting activity as competitive antagonists of estradiol in uterine tissue are useful as antifertility agents because they may be devoid of estrogenic activity or have a large separation of antifertility and estrogenic activity. A series of steroidal compounds, synthesized or obtained from other sources, are to be tested as competitive inhibitors of estradiol against the 8S and 5S receptors and then tested for antifertility activity in rats, rabbits, and hamsters.

Major Findings: A large number of compounds were tested for ability to compete with ^{3}H-estradiol for binding in the nuclear and non-nuclear fraction of rat uteri 2 hours after s.c. injection. A large number of estrogen analogues were tested for anti-implantation and estrogenic (i.e. uterotrophic) activity. Of these, 16-epiestriol exhibited the highest anti-implantation to estrogenic ratio. The effect of 16-epiestriol on estradiol binding was in the intermediate range. Estriol was also found to interfere with the binding of estradiol.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of steroidal compounds for contraceptive utility.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthesizing and Testing of Antifertility Compounds
Contractor: Collaborative Research, Inc.
Money Allocated: $137,316 (FY 71) for two years

Objectives: It is the purpose of this project to synthesize a series of polyheterocyclic compounds and to test their antifertility effect in mice and rats.

Major Findings: The synthesis of some of the polyheterocyclic compounds has been completed. Major biological findings are not available at this early stage of development.

Significance to Biomedical Research and Program of the Institute: The work undertaken to synthesize these polyheterocyclic compounds and test them for antifertility activity is directly relevant to the purposes of the Contraceptive Development Branch activities.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Synthetic Antifertility Compounds
Contractor: Stevens Institute of Technology
Money Allocated: $52,301 (FY 71)

Objectives: It is the purpose of this project to synthesize a series of polycyclic, polyheterocyclic, and steroidal compounds and to test their antifertility effect in rodents.

Major Findings: The synthesis of several of the required polyheterocyclic compounds has been completed. Major biological findings are not available at this early stage of development.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of steroidal and non-steroidal compounds for contraceptive utility.

Proposed Course: Termination

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Contraceptive Development Branch
Contract and Collaborative Research

Contract Title: Contract Program for Obtaining, Synthesizing, and Testing Antifertility Compounds
Contractor: Hazleton Laboratories
Money Allocated: $44,800 (FY 72)

Objectives: The purpose of this project is to study and confirm the antifertility activity of an antibiotic mixture in dogs and several species of small laboratory animals and to identify the active agent.

Significance to Biomedical Research and Program of the Institute: The work undertaken in this project is directly relevant to the purposes of the Contraceptive Development Branch to support the synthesis and evaluation of novel chemical agents for contraceptive utility.

Proposed Course: This is expected to be a continuing contractual effort leading toward the development of new chemical contraceptives and is an integral part of the contraceptive development program.

NICHD Annual Report
July 1, 1971 through June 30, 1972

Population and Reproduction Grants Branch
Center for Population Research

An inventory of all aspects of reproductive processes in experimental animals and humans is the foundation from which significant improvements in family well-being and fertility control, as well as in approaches to the problems of sterility will develop. The Center for Population Research provides most of the support and direction of this broad field, and it is imperative that efforts in support of this essential element of a population research effort be continued.

The Population and Reproduction Grants Branch within the Center for Population Research has the responsibility of administering all basic research and training grants approved for funding by the research grant mechanism in the biological, clinical, biomedical and social (behavioral) areas relating to reproduction and population phenomena and aimed at gaining a fuller understanding of normal and abnormal reproductive and early developmental processes in animals and human beings. It is concerned with all aspects of population fertility and infertility, particularly studies likely to yield new information and methods for the development of safer, more acceptable contraceptives, as well as with the training of young investigators to accelerate and broaden the research effort. The mission of the Branch has been broadened with the initiation of the new Population Research Center Award Program, which currently is funding five such Centers. Seven fully functional Centers are anticipated within another year.

As of March 1972, grant funding of $17.5 million supports 275 research grants, 30 training grants, 12 fellowships and 28 research career development awards. This funds research in the area of reproductive biology and contraceptive development, animal behavior and ecology, and the social aspects of population research. In biomedical research the scope of current funding at $9.5 million is indicated by the topics:

A. Morphology and associated function of all tissues involved in reproduction

B. Mitosis and meiosis in gamete formation

C. Gamete transport and fertilization

D. Pre-implantation differentiation

E. Biochemical studies of semen, and tubal fluids

F. Synthesis and physiological function of all hormones involved in reproduction

G. Localization and identification of steroid and protein binding sites

H. Pheromone and endocrine control of sexual behavior

I. Gonadotropin releasing-factors and their control of reproductive processes

J. The influence of environmental variables and population density on reproduction

During FY 1972, staff worked to expand and diversify the behavioral-social science population research grants program while attempting to obtain a balance of substantive research areas. The program continued to grow during 1972; the increase in number of proposals by fiscal year is as follows: FY 1970 - 25; FY 1971 - 125; FY 1972 - 151. The first ad hoc Special Study Section, meeting in May 1969, evaluated eight proposals, while in FY 1972, the Study Sections averaged 50 proposals a meeting. The permanent Population Study Section established to replace the ad hoc Sections met for the first time in January 1972.

Behavioral-social scientists from a variety of disciplines have been stimulated to submit research grant proposals. While very much still needs to be done, it is worth noting that the proposals received in FY 1972 were submitted by members of 21 different disciplines. The disciplines were represented as follows: Psychology - 24%; Sociology - 16%; Economics - 16%; Demography - 12%; Anthropology - 7%; Psychiatry - 7%; Political Science - 3%; Geography - 2%; Social Work - 2%; Communications - 2%; and Home Economics - 1%. The following disciplines submitted one proposal each: Public Health, Population Genetics, Statistics, Engineering, History, Epidemiology, Physics, Obstetrics-Gynecology, Pediatrics, and Physiology. While much work needs to be done with all the disciplines, Anthropology, Geography, and Political Science may be singled out as social sciences which could engage in more population research and submit more proposals.

The behavioral-social science proposals received in FY 1972 proposed to study a broad range of population phenomena. As in FY 1971, studies of fertility still constitute the major part of the proposals, while investigations of population policy are the smallest part. A classification of the FY 1972 funded proposals indicates that they are in the following substantive areas:

A. Psychological factors related to fertility and family planning

B. Family formation, fertility, and out-of-wedlock pregnancies of pre-adults

C. Problems of abortion

D. Demographic studies of fertility

E. Economic factors in fertility

F. Familial, role, and life style factors in fertility

G. Population growth and distribution

H. Population policy

The mission of the training grants program relates directly to the major aspects of basic reproductive biology and contraceptive development, as well as the behavioral-social sciences. Specifically, it fosters and supports research training in the following areas: reproductive physiology and behavior and their disorders; the behavioral and biomedical aspects of fertility; the biological, economic and psychosocial consequences of fertility regulation and experience, and their interaction with other demographic variables; the development of population policy; normal and abnormal development of the pre-implantation zygote; and implantation and placental induction.

By the end of Fiscal Year 1972, it is expected that approximately 250 pre- and postdoctoral trainees will be in training; approximately 2/3 of these are supported on a full-time basis. From data available from progress reports for FY 1964-1967 on about 100 past trainees, it is estimated that 92% of full-time trainees go into teaching and research, 3% enter clinical practice, 1% accept positions in industry, and 4% take positions in other areas or enter the armed forces.

During Fiscal Year 1972, 18 training grant applications were reviewed by the Population Research Training Committee, of which Dr. Bengt Liljeroot is Executive Secretary. Of these, 8 were new applications and 10 were competing renewals. A total of $1.5 million was requested by the applicants. The committee recommended approval of 6 new applications for $257 thousand, and 8 competing renewals were recommended for continuation, for $610 thousand. Of these only 1 new training grant and 2 renewals were awarded, for $149 thousand.

However, 4 competing renewals reviewed in Council Year 1971 were also funded. It is expected that 14 noncompeting renewals totaling $1.5 million will be awarded by the end of FY '72. Total FY '72 training grant awards (including indirect costs) are estimated at about $2.1 million. In all, 25 training grants are expected to be funded by the Center for Population Research. Of these, 10 are for training in biological science departments, 9 for training in behavioral and social science departments, and 6 for research training in clinical departments.

The Center for Population Research also supports research training through a Postdoctoral and Special Fellowship program and the Research Career Development Award. During Fiscal Year 1972, $764 thousand was available for RCD awards and $650,000 for fellowships.

During this year, several staff changes have occurred in the Branch. Dr. Everett Wilson, a reproductive biologist from Sam Houston University in Texas, joined the Center in June 1971 as Branch Chief. Dr. William Sadler, Texas Southern University, has been assigned to the staff as a scientist administrator where he will share the work and responsibilities with Dr. Allyn Waterman in the biomedical area. Dr. Sadler joined the staff in May 1972.

Mrs. Thanora Coleman joined the staff as clerk-typist in July 1971, where she helps to minimize the work load in the population and reproduction and training program areas. Three scientists enrolled in the Grants Associate Program, National Institutes of Health, spent three to six weeks in the Branch during this year: Drs. George Willis, Jay Moskowitz, and Omar Yoder, and intramural researcher Charles Cargille. They participated in the daily work of the branch, and completed a program assignment designed to assist them in developing a career in health science administration.

On July 1, 1970, the Center for Population Research, announced a new program to establish Population Research Centers in order to provide core support and new program development for non-profit organizations conducting research and training relevant to population problems. This program represents a commitment by the National Institutes of Health to the long-term support of institutions with demonstrated ability to conduct research and research training in areas that seem likely to hasten progress toward solving problems related to human population growth, structure, and distribution. Population Research Centers are designed to facilitate multidisciplinary communication and effort in the population field, give visibility to the research area, and provide administrative support and a physical environment conducive to scientific productivity. Ongoing Centers have been established at: (1) The Population Council, New York City, Biomedical Division, Dr. Sheldon Segal, Director; (2) University of Texas, Population Research Center, Dr. Harley Browning, Director; (3) Vanderbilt University, Department of Obstetrics and Gynecology, Dr. Bert O'Malley, Director; (4) University of Wisconsin, Center for Demography and Ecology, Dr. Halliman Winsborough, Director; (5) Harvard University Medical School, Center for Population Research, Dr. Roy Greep, Director.

Program Project Grants, in contrast to Population Research Center Grants, provide research support for the investigators as well as core facilities. During this year the Program funded nine program projects; four of these were new awards. These awards encompass all aspects of population research, both clinical and non-clinical, biomedical and behavioral. These are located at: Columbia University (Dr. Vande Wiele); University of Hawaii (Dr. DeFeo); Johns Hopkins University (Dr. Biggers); University of Michigan (Dr. Midgley); Ohio State University (Dr. Van DeMark); Oregon Primate Research Center (Dr. Montagna); University of Pennsylvania (Dr. Mastroianni); and University of Texas at Austin (Dr. Jacobson).

The Population and Reproduction Grants Branch has administered as one aspect of its program the development of conferences, symposia and workshops to assess the present state of knowledge of selected areas of basic reproductive biology deemed particularly pertinent to future contraceptive development, as well as to identify areas where imaginative approaches may accelerate research development and progress. A further purpose is to identify and encourage investigators who may not now be actively working in the field so that interdisciplinary exchange may be effected and rapid progress may be made in solving the complex problems of basic human reproduction.

During this year a publication "Oogenesis", edited by Dr. John Biggers and Allen Schuetz, presented the result of a symposium in reproductive physiology organized by Drs. Biggers and Schuetz and held at the Johns Hopkins University Department of Population Dynamics, October, 1970.

The book combines a systematic interdisciplinary comparative review of the present state of the topic of oogenesis and emphasizes mechanisms of meiosis, developmental aspects of information storage, the cellular vehicle and nutrients necessary for development, and the endocrine regulation of oocyte maturation and release, along with the most recent experimental and methodological developments and suggestions for future research. It is a companion volume to other symposia, held in previous years; important topics that have been discussed previously include: fetal homeostasis, comparative reproductive failure, the mammalian oviduct, biology of the blastocyst, and biology of the cervix.

Another topic of interest to the Branch was the subject of a symposium with accompanying workshops organized by James Leathem, Ph.D., Director, Bureau of Biological Research, Rutgers University, entitled "Immuno-reproduction," and held in conjunction with the annual meeting of the Society for the Study of Reproduction at Boston University, June, 1971. This symposium continues a series supported by the National Institute of Child Health and Human Development. Previous topics in the series have included: male reproduction, gametes and fertilization, and environment and reproduction. The proceedings in each instance have been published in the journal of the Society. The purpose was to review the phenomenon of immunology as it relates to reproductive processes in both male and female, present the subject of immunoreproduction to reproductive physiologists with varied backgrounds and thus to touch many areas, to inform them of the present status of the field, to provide new research data and to probe the potential of future research, and to examine the role of immunology in the infertile human. Presentation of the potential for research in reproductive control may invite new investigators and new concepts.

Behavioral-Social Science Workshop. A behavioral-social science workshop on Developing and Educating Psychologist for Work in the Population Area was supported by the Center for Population Research, and held at the University of North Carolina on October 24-26, 1971. The Center and the American Psychological Association Task Force on Population, Family Planning, and Population Policy were co-sponsors of the Workshop. Participants included six chairmen of departments of Psychology, sixteen additional psychologists important in graduate training, and the members of the APA Task Force. The Workshop explored problems of motivating and educating Psychology students, at both the graduate and undergraduate levels, to enter and work in the population field.

A full report of the second workshop on abortion entitled "Abortion, Obtained and Denied: Research Approaches", held in December, 1969, and jointly sponsored by the National Institute of Child Health and Human Development and the National Institute of Mental Health, was published as a monograph by The Population Council in 1971.

fertilization. Infection of mammalian spermatozoa by a virus (purified simian virus 40, SV40) may be a method of carrying genetic material into an egg in order to study the fate of the embryo developing from treated ova. Further development of these techniques will aid in the study of gene control of neoplasias, such as mammary tumors and hepatomas, and of genetic abnormalities.

Probably one of the most popular issues today is the role of adenyl cyclase and cyclic AMP on hormonal-cellular interactions. Recent findings coming out of Dr. O. Hechter's laboratory provide suggestive evidence that cyclic AMP is involved not only in the action of gonadotropic hormones but also of testosterone as well as prostaglandins upon target cells in the ovary and testis. The adenyl cyclase -- cyclic AMP system may, therefore, be an extremely useful system for investigating certain aspects of the control mechanisms associated with differentiation of germinal cells primarily in the testis. Cyclic nucleotide systems may be very important in the regulation of metabolism, the entire process of spermiogenesis, and motility and fertilization capacity of spermatozoa. There is evidence that adenyl cyclase is an active intermediary between LH and the corpus luteum (March, 1970). Center for Population Research is supporting several grants in which the central theme underlying the research projects is the hypothesis that cyclic AMP, and perhaps other cyclic nucleotides, play a key regulatory role as a mediator in the informational interchange which occurs between the cell and chemical signals in the environment be they hormones, enzymes or drugs. This is not a new hypothesis, but one which has been elaborated on by Dr. Earl Sutherland for which he won a Nobel prize.

The culture of cells in vitro, whether somatic or reproductive, provides the investigator with a tool that can be utilized in basic studies of hormone action at cellular and subcellular levels. Recent advances have been made in the culture of specific cell types such as the granulosa cells from the ovarian follicle (Channing). Since these cells undergo luteinization and steroidogenesis in culture, the action of gonadotropic hormones as well as their steroidogenic activity during the stages of the menstrual cycle can be analyzed. Culture of cells also aids in the study of histogenesis and cytodifferentiation as these processes occur in vitro. Similar work is being done with fetal mouse ovaries particularly with reference to the development of the follicle (Odor and Blandau, 1971). This technique also lends itself to the study of the young embryo, e.g. cleavage stages to blastocyst implantation (Iwamatsu and Chang, 1971).

The key to a complete medium for in vitro culture, particularly of 2- or 4-cell stages to the preimplantation blastocyst, has been the discovery that pyruvate is the central energy source for mouse and rabbit embryos, even for maturation stages of the unfertilized ovum. Since this discovery, significant advances in the in vitro study of ova, sperm and the developing zygote have been made (Brinster).

Even though there has been much progress made in mammals it is only in recent years that the problem has been approached in man. Dr. L. Mastroianni and associates at the University of Pennsylvania School of Medicine have developed an in vitro system in which human ova may be matured, fertilized with human sperm after incubation in the uterus of a midcycle rhesus monkey, and

followed in their development through early cleavage stages. While cleavage was the end point sought in these experiments, (2-12-cell stages were found) and one ovum with two polar bodies was seen, this study, as the investigators state, does not rule out spontaneous development without fertilization. Spermatozoa were observed adherent to and within the zona pellucida, and sperm mobility was noted to persist for 3 days under these experimental conditions. This study differs from other reports of an in vitro fertilization of human ova in that ova from mature follicles were selected and no preceding hormonal (gonadotropin) treatment had been employed. The aim was to develop a repeatable system. An understanding of in vitro conditions under which human ova are activated will afford the scientist opportunities to evaluate factors which retard as well as enhance fertility.

Dr. Lawrence L. Espey, Trinity University, has been interested in determining how a mature ovarian follicle, which is destined to rupture, responds to a preovulatory surge of luteinizing hormone (LH) by liberating the contained ovum. Ovulation is not caused by an increase in intrafollicular pressure. Adrenergic nerves and smooth muscle tissues are present, and may be involved in the process (Visutamasen, et.al.). Recent data suggests that ovulation involves active decomposition of the massive collagenous tissue in the follicular wall, presumably requiring proteolytic enzyme action. Dr. Espey has recently shown that ascorbic acid metabolism in the ovary changes in response to LH by reducing the tensile strength of the follicle. The fibroblast within the follicular wall may be the source of the enzymes which cause collagenolysis, and he has found evidence in his study of the ultrastructure of such fibroblasts to indicate that unique multivesicular structures, protruding from cell surfaces, appear to digest the extracellular matrix in the thecal layers. These vesicles appear to contain acid phosphatase, and they are probably not related to lysosomal bodies.

The mechanism of action of the intrauterine device (IUD) remains unclear. It may provoke changes in blastocyst metabolism and alter the adhesiveness of the trophoblast. The effectiveness appears to be correlated with the amount of the area of contact between endometrium and IUD, and the inflamatory reaction is characterized by leucocytic infiltration into the uterus and a changed milieu. The addition of copper to the IUD or the use of a copper IUD has been shown to contribute to effectiveness. Even a small length of copper wire within the uterine horn or a copper ring transfixing the uterine lumen decreases the number of implantation sites. The antifertility effect is limited to the treated horn (Chang and Tatum, The Rockefeller Institute). Furthermore a "copper-influenced" blastocyst can be transferred into a normal uterus where it will implant and develop typically, whereas a normal blastocyst cannot implant when it is transferred into a uterine form which has been "influenced" by copper. Inhibition occurs only when the copper wire is placed in the uterine horn prior to implantation. It appears that ova transported through the oviduct are not affected since normal implantation occurs in both treated and control horns when a copper wire is left in situ until, but not including Day 5 of pregnancy (Chang and Tatum, 1972). There is a critical time period for the action of a copper IUD (ring), and this period is around 48 hours if the 4th day of pregnancy is included. The uterus is very sensitive to any mechanical disturbance on the 4th day. Experiments seem to show that the mechanism of contraceptive action of metallic copper may be independent of any change in the endometrium brought about by exogenous ovarian steroid hormones.

The ubiquitous prostaglandins are being investigated as potential fertility interrupters. Two natural ones may be good abortifacients, but do not seem superior to oxytocin as inducers of labor. Side effects are serious deterrents at least at the dosages reported, but perhaps the new ones being synthesized may be more efficacious. The best mechanism of administration remains to be determined. Sooner or later there may become available to anyone an abortifacient pill to be taken by the individual in early pregnancy; this would only need to be done at most three times per year thus doing away with the need of loading the human system with high hormonal levels. Such a contraceptive could be world wide in its acceptance in any society or economic level.

Subcutaneous injection of prostaglandin F2a (PGF2a) prevents pregnancy in hamsters and blocks nidation in rat and rabbit; and along with intravenous infusion of E-2 or F-2 terminates pregnancy in the rhesus monkey. Therapeutic abortion has been reported. When injected subcutaneously in days 3, 4 and after insemination or implanted subdermally in silastic tubes on day 3 after insemination, PGF2a in concentrations of 0.5 to 2mg (glossy material, pure compound, or methoxamine salt) had no effect on implantation and embryonic development. Development was affected when the prostaglandin was injected for days 3-7. Injection of 2 mg/kg/ day in days 8-10 soon after implantation caused complete degeneration of embryos. 5 mg injected on days 3-7 reduced the number of corpora lutea, and inhibited implantation significantly and embryonic development completely. Injected PGF2a at 5 mg/kg in either day one or two prevented implantation significantly, no eggs were found in the reproductive tract, but not embryonic development after implantation; whereas injection in day 4 or 6 had little effect in either. (Drs. Chang and Hunt, Worcester Foundation for Experimental Biology). Given in day 21, all fetuses were aborted. With 0.5-2 mg/kg egg transport was little affected. Many corpora lutea are smaller and not distinctive following treatment. PGF2a is also quite toxic.

Prostaglandin PGF2a is known to stimulate contraction of the oviduct and uterus in vivo and in vitro, whereas in the rabbit PGE2 reduces oviductal motility, relaxes the isthmic circular muscle and reduces its opening pressure. Induction of luteolyses has been reported. Testicular contractions in rabbits can be modulated in vitro by prostaglandin PGF (Dr. Johnson, et.al., Utah State University). This suggests the possibility of in vivo control by prostaglandins that may be synthesized in the testis, in addition to acetylcholine and norepinephrine as postulated by other investigators. When the prostaglandin was added to the bathing medium (Tyrode solution), an increase in rate and overall tone with a change in amplitude was seen in the autorhythmical contraction; lower concentrations caused an increase in amplitude, lower levels provoked a decrease.

During fertilization in mammals and non-mammalian species the interaction of the fertilizing spermatozoon with the vitelline membrane enclosing the egg induces a change in the character of the egg surface and the egg is also stimulated to continue its interrupted development. The zona pellucida around the egg changes so that penetration by further spermatozoa is greatly reduced. Several investigators attribute this to the material released by cortical granules into the pervitelline space (Drs. Barros and Yanagimachi).

The surface reaction also apparently renders the egg plasma membrane refractory to the entry of other spermatozoa and provides a block to apolyspermy. Interest continues in these changes in the egg surface. When fertilized and unfertilized rabbit eggs devoid of the zona pellucida were exposed at pH 1.8 to positively charged ferric colloid particles, the vitelline surface of the fertilized egg bound more of these electron-dense particles. Thus one of the changes at the egg surface involves an increase in the concentration of negatively charged groups, contributed by carboxyl radicals of surface sialic acids present in the N-acetyl-O-diacetyl configuration (Bedford and colleagues, Columbia University College of Physicians and Surgeons).

When the hamster spermatozoon passes through the egg investments (cumulus oophorus and zona pellucida), multiple unions between the plasma and outer acrosome membranes take place and the contents of the acrosome cap and the acrosome collar are released. The major active substance released from the acrosome cap is believed to be hyaluronidase (cumulus oophorus-dispersing enzyme) and that from the acrosome collar to be an enzyme to dissolve the zona pellucida (zona lysin). The perforatorium, which does not undergo any significantly visible changes at the time of sperm passage through the egg investments, appears to function as a mechanical tool whereby the spermatozoon cuts through the material of the zona pellucida. The postnuclear cap region of the spermatozoon obviously plays an important role in the initiation of the membrane fusion between the spermatozoon and the egg (Dr. Yanagimachi and colleagues, University of Hawaii School of Medicine). On the basis of these findings, a model of the sequence of events in fertilization is being developed.

These investigators have also found that when the hamster spermatozoon reaches the surface of the egg, numerous microvilli of the egg surround it. Those microvilli attached to the acrosomal collar region of the spermatozoon quickly spread over the inner acrosomal membrane of this region, dislodging the outer acrosomal membrane and the overlying sperm plasma membrane. At the same time, microvilli fuse with the plasma membrane of the postnuclear cap region of the spermatozoon, and the egg cytoplasm quickly flows around the nucleus of that region of the spermatozoon. The inner acrosomal membrane persisting on the sperm head never fuses with the egg (microvillar) plasma membrane. This membrane is destined to disintegrate and be absorbed into the egg cytoplasm. Incorporation of the sperm flagellum into the egg cytoplasm takesplace by fusion between plasma membranes of the spermatozoon and egg.

In order for the sperm to penetrate the cellular cumulus oophorus and noncellular zona pellucida membrane enclosing mammalian eggs, at least in some species, the sperm must undergo changes (capacitation) that are provoked by the uterine and oviductal environments through which spermatozoa pass before reaching the eggs in the oviduct. Studies both biochemical and structural by reproductive physiologists continue to be pursued in order to discover these changes (Dr. Bedford, Columbia University College of Physicians and Surgeons; Dr. Williams, University of Georgia). Other studies relate to factors provoking capacitation, or decapacitation, species variation, time following coitus, etc. Such knowledge is vital toward the understanding of the successful accomplishment of fertilization in vitro of human eggs, with

possible application in the future in cases of human genetic abnormalities and certain types of sterility.

Dr. M. J. K. Harper, Worcester Foundation for Experimental Biology, continues his studies of factors influencing sperm penetration of rabbit eggs. It is clear that unfertilized eggs destined for fertilization in the oviduct of a recipient should be transferred as soon as possible (question of hours) after ovulation. This should be done before the cumulus cells are shed. However, after a number of hours, for example, it makes no difference in fertilizability whether cumulus (plus even the corona) is present or not. Apparently, when conditions for fertilization are not optimal, the presence of the cumulus is essential. Also the products of ovulation play no role in the final stages of capacitation of the spermatozoa.

The relation of the cortical reactions in the egg, resulting from sperm penetration, has interested Drs. Longo and Anderson. Their studies were performed on the sea urchin egg as an animal model. Employing eggs narcotized with urethane to produce polyspermy, they have demonstrated that the release of the cortical granule material of activated eggs plays no significant role in the events following fertilization. This material is restricted to the cortical reaction at fertilization, and is not related to aspects associated with the pronuclei. Polyspermy can also be induced by treating sea urchin eggs with nicotine. The number of spermatozoa entering the treated eggs is related to the concentrations of nicotine employed. Mitosis and cleavage of these polyspermic zygotes occur later than in monospermic eggs. Results indicate that the events of fertilization are affected primarily quantitatively.

If the cleavage embryo is transported too swiftly from its site of fertilization in the oviduct to the uterus, neither the embryo nor the uterine environment are sufficiently prepared for implantation, and the embryo dies. Failure of fertilization can result from too few spermatozoa reaching the mammalian egg. Recently the effectiveness of several low-dose synthetic progestins as oral contraceptives (megestrol acetate, melengestrol acetate, chlormadinone acetate) has been attributed to their effect on gamete transport. Drs. Chang and Hunt, Worcester Foundation for Experimental Biology, have reported that the number of sperm recovered from the uterus was lower only in progesterone - treated rabbits, whereas the number in the oviduct was lower in progestin-treated animals. The proportion of fertilized eggs was lower, and of embryos reaching the uterus was higher, in animals treated with progesterone, nongestrel and chlormadinone acetate as compared with controls. Fertilization of mammalian eggs in vitro is now a reality, and factors involved in this process are being enthusiastically studied for possible application to the human.

Preovulated mouse eggs can be penetrated by epididymal spermatozoa but the block to polyspermy (zona reaction) is weaker than for eggs recovered just before or after ovulation. Polar bodies may fail to form (Drs. Iwamatsu and Chang, Worcester Foundation for Experimental Biology). Fertile life of mouse eggs lasts about 10 to 12 hours after ovulation. In aged eggs there occurs a failure of the transformation of sperm heads as well as activation of eggs. The presence of epididymal extract appears to inhibit sperm penetration. The

concentration of spermatozoa, concentration of calcium ions, and the pH of the medium can all affect the capacitation of mouse spermatozoa and the penetration of mouse eggs in vitro. Dr. Brackett, University of Pennsylvania, has found no differences in the sequence of events of fertilization and early cleavage between rabbit ova fertilized in vivo and those fertilized in vitro.

There is the possibility that animal viruses can be used to transfer genetic information or repair genetic defects. While mammalian ova, both unfertilized and fertilized, have been infected with virus in studies of DNA transfer, only recently has this been accomplished in mammalian spermatozoa. Apparently the spermatozoon does not permit penetration of the SV40 virus, unlike somatic cells of the same species, but does take up SV40 DNA which could be recovered from spermatozoa after fusion with SV40 susceptible somatic cells. This becomes localized in the post-acrosomal area of the spermatozoan head. (B.G. Brackett and colleagues, University of Pennsylvania and The Wistar Institute of Anatomy and Biology). Biologically active SV40 DNA has also been recovered from fertilized rabbit ova, indicating a transfer by infected spermatozoa during fertilization. This is the first recorded instance of such an event taking place in the mammalian organism. It is not possible as yet to speculate on the degree of incorporation or integration of heterologous DNA into spermatozoa. Further exploration of such a transfer through the process of fertilization is being made along with observation of the fate of the embryo developing from treated ova.

As the channel between the ovary and the uterus, the mammalian oviduct continues to interest investigators of reproductive physiology. Within this tube gamete transport takes place to site of fertilization, fertilization itself occurs and during the following few days the young embryo is moved to the uterus where it arrives as a ball of cells enclosed within the zona pellucida membrane. Motility characterizes this important tube, as well as changing fluid content and ciliary degeneration and subsequent regeneration all correlated with phases of the reproductive cycle and necessary for gamete and embryo transportation. While the role of estrogen and progesterone in control of oviductal secretions is well established, there have been few studies with autonomic drugs. Dr. Brunton has investigated the effects of adrenergic agents on transmembrane potential (PD) and short circuit current (SCC) of an isolated preparation of rabbit oviduct. Results indicate that PD and SCC of rabbit oviduct are stimulated by adrenergic agents. This stimulation is associated with beta receptors in the oviduct; cyclic 3^1, 5^1-adenosine monophosphate (cyclic AMP) and theophyllinc mimic this effect. The sensitivity of these receptors to low levels of catecholamines suggests that they play a role in regulating oviductal secretion. If beta adrenergic stimulation or increased cyclic AMP levels accelerate the rate of active transport of chloride by the oviduct, this would be consistent with the electrical effects he observed. Whether regulation is mediated primarily by the adrenergic innervation or by circulating catecholomines, or whether the sensitivity of these receptors varies with reproductive state, has not been determined.

Motility of the rabbit oviduct has been studied by Salomy and Harper, Worcester Foundation for Experimental Biology, by means of indwelling, balloon-ended, fluid-filled polyethylene catheters inserted through the

uterine muscle to terminate near the uterotubal junction or through the junction into the oviduct. The rabbit oviduct is characterized by bursts of activity lasting for 2-3 minutes, or sometimes longer, followed by quiescent periods ranging up to 20 minutes. There appears to be no correlation for bursts of activity in the two oviducts of an animal. Daily variations of activity may occur, as well as peaks of activity every 4-7 days. Each oviduct exhibits its own pattern of cyclic activity. Following the hormonal induction of ovulation, increased activity may occur after about 4 hours, by which time bursts of contraction occur every 15 minutes. This is followed by a rapid increase in the mean amplitude of contraction until 10 hours, and in turn by a further slower increase up to 24 hours after injection. At this latter time the bursts of activity occur every 5-8 minutes. A slow return to the amplitude characteristic of the preinjection period takes place in the next 2 days.

The fluid in the lumen of the mammalian oviduct appears to be derived mainly from the oviductal epithelium itself. The composition of this fluid as well as its rate of formation in the rabbit, for example, change at the time of ovulation, during pregnancy, after castration and following injection of the ovarian hormones. The mechanisms involved in the production of this fluid are not clearly understood. Drs. W. J. Brunton and R. L. Brinster, University of Pennsylvania School of Medicine, have studied specific ion transport processes of the epithelium by applying a short-circuit current technique to the isolated ovarian end of the oviduct of the rabbit. Results show that the movement of Na is consistent with passive diffusion whereas the net flux of Cl is coupled to an active transport mechanism. This work is performed by the oviduct to cause a net movement of Cl into the mucosal solution. The transmembrane potential and short-circuit current were reduced or abolished by cyanide, dinitrophenol, ouabain or anoxia, which indicates that the process responsible for the net flux of Cl is dependent on energy derived from oxidative metabolism.

Contrary to the generally accepted position that smooth muscle of the reproductive tract exhibits maximal contractility only when under the influence of estrogens, Drs. Boling and Blandau, University of Washington School of Medicine, have presented data recently that implies that in the presence of physiologic concentrations of estrogens the muscle is minimally active; that activity increases as estrogen content decreases; and that oviductal muscle subject to the influence of estrogens becomes increasingly active when progestins are introduced. These investigators have studied the patterns of muscle contractions of the ampullae of the rabbit oviduct which support this novel concept, as well as ovum transport.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

The program to evaluate medical effects of fertility regulating methods was characterized by rapid expansion during FY 72. Four Requests for Proposals were issued on the following subjects: Comparative Pharmacology of the Contraceptive Steroids (RFP 72-2), Evaluation of the Long Range Effects of Vasectomy (RFP 72-4 and 5), and Evaluation of the Interaction of Contraceptive Steroids and Essential Dietary Nutrients (RFP 72-6). During the year, five review panels were assembled to consider unsolicited proposals, proposals submitted in response to the Requests for Proposals, as well as renewal programs. In July 1971 proposals to develop abortion surveillance programs both in this country and abroad were considered by a panel; other panels met in October 1971 to review the Comparative Pharmacology proposals, in March 1972 to evaluate proposals in response to RFP's 72-4,5 and 6 and in April 1972 for evaluation of programs for renewal. In addition, three workshops were held to evaluate and plan program activities. In July 1971, a workshop to plan the program on nutritional status of contraceptive users was conducted and workshops for investigators conducting studies on the relationship between hormonal use and breast cancer were held in October 1971 and April 1972. The staff of the branch was expanded by the addition of one professional and one clerical member. The budget which will be required to fund the approved programs for FY 72 will be nearly double that requested in FY 71.

With regard to new activities, the program to evaluate the comparative pharmacology of the contraceptive steroids will place the required preclinical testing procedures to screen new and marketed contraceptive steroids for the possibility particularly of carcinogenic as well as other effects on a firmer scientific base. Improvement of these protocols will ease in interpretation and facilitate the introduction of new compounds. The program concerning nutritional status of oral contraceptive users will expand present knowledge about the already defined changes in nutritional biochemistry which are produced by the use of the contraceptive steroids. If these effects are significant, they may be corrected readily by the addition of vitamins or minerals as a dietary supplement. Finally, the program to evaluate the long range effects of vasectomy using epidemiological and laboratory techniques will determine whether presently limited information suggesting that standard vasectomy procedures might be associated with local effects on the testis affecting endocrinologic status, or systemic effects on the general immunologic system, should influence the already increasing use of male sterilization in the United States.

The ongoing study of the cohort of more than 10,000 women under multiphasic evaluation at the Kaiser Foundation Hospital, Walnut Creek, California, continued. During the past year, there was emphasis on having patients return for repeat evaluation and also to complete both cross-sectional and longitudinal analyses of all the components of the screening technique. This evaluation will lead to modifications in the testing procedure as well as a

shifting emphasis to surveillance during FY 73. A large number of reports have now become available all of which have either been submitted or are accepted for publication including the following: blood pressure, blood coagulation, blood cell indices, plasma proteins, pulmonary function, glucose tolerance and behavioral effects. Preliminary results indicate a modest but definite increase in blood pressure in women using oral contraceptives as well as increased activity of coagulation function, decreased glucose tolerance, various changes in the plasma proteins but no effects of the drugs on pulmonary function.

Studies of steroid metabolism begun during FY 72 to evaluate relationships between the dosage, unique properties of drugs and various pharmacological effects continued as planned. Included are studies of the absorption, metabolism and distribution of the various steroids being conducted by several investigators (Williams, Mahesh and Horning). A study of the dose-response relationship of various combinations of estrogens and progestins with regard to pituitary-ovarian function was initiated (Schalch). Finally, an assessment of possible abnormal metabolic patterns in women using oral contraceptives as well as the influence of other drugs on the metabolism of the oral steroids was begun (Bressler). These studies are underway and results should become available during FY 73.

A group of studies in women with breast cancer and cervical dysplasia have been continued (White, Paffenbarger and Stern). Preliminary analyses on each of these studies reveal no risk of either breast cancer or progression of cervical dysplasia as related to the use of the contraceptive steroids. A second multi-clinic prospective study of the relationship between abnormal cervical cytology and contraceptive use was launched during late FY 72 after completion of data collection instruments and logistic planning (Temple Univ.). Because of the long latent period between exposure to a cancer-causing agent and diagnosis of the disease, it is anticipated that projects presently under support will provide a nucleus for long-term monitoring for the occurrence of these conditions.

Studies have been underway to further refine the mechanism and risk of thromboembolism in women using oral contraceptives. Data collection in a study of stroke in women of reproductive age has now been completed and is undergoing analysis; on the basis of preliminary findings, an increased risk of stroke in women using oral contraceptives is supported but further information relating to age, ethnic group, drug used or other predisposing factors awaits completion of the analysis (Heyman). An additional multiclinic study of thromboembolism will furnish additional information about other sites of this disease as related to risk factors and drug dosage (Stolley). Procedures which may be able to predict impending thrombotic events in women using oral contraceptives continue to be under development (Fletcher). Meanwhile, studies in monkeys to evaluate the effects of steroids on the blood vessel wall and blood flow are nearing conclusion (Loeb). The rise in blood pressure found in many women using contraceptive steroids has been explored systematically in a study in which individual drugs and detailed physiological evaluation has been conducted (Crane); the study is nearing completion and data should be available during FY 73.

A study of the effects of oral contraceptives on glucose metabolism in women with diabetes or at high risk of developing diabetes is continuing (Gershberg). Preliminary evaluation of the results suggest that the oral contraceptives are not detrimental to insulin-dependent diabetics but have more significant effects on the latent diabetic or obese group.

There has been continuing concern that the use of the steroid contraceptive drugs may produce genetic effects in the offspring of women having used this contraceptive method. A longitudinal study has revealed a slight but significant increase in chromosome breakage in lymphocytes of oral contraceptive users (Littlefield); further evaluation of the significance and mechanism of this effect is now being planned. Continuation of a study of chromosomes in the ova and abortuses of women having used steroid contraceptives is being continued after prior support from the Contraceptive Development Branch (Klinger); this study will give more direct evaluation of genetic effects on germ cells. In addition, a study in a local military hospital of the outcome of pregnancy in women exposed to contraceptive steroids has been begun and will serve as a model system for initiation in other collaborating institutions in the near future (Bailey).

The evaluation of other contraceptive methods such as the intrauterine device (IUD) and abortion has continued. A study of the physiological effects of IUD's in large animals and the influence of various drugs which affect liver enzymes and reproductive physiology has continued to be productive (Hawk). An abortion surveillance program based at the Communicable Disease Center, Atlanta, Georgia, obtaining information from hospitals, state and local health departments was continued during FY 72 (Tyler).

A one-day symposium to present the current status of techniques of chemical contraception will be conducted as part of the Vth International Congress on Pharmacology (Fed. Am. Soc. Exp. Biol. Med.). Subsequent to the symposium, a two-day workshop on the steroid contraceptives will be sponsored in conjunction with the Drug Research Board, National Academy of Sciences; a series of reports on the status of selected research subjects will be prepared by the various work groups.

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Fertility Regulating Methods Evaluation Branch

Contract Title: Contraceptive Drug Study
Contractor: Kaiser Foundation Research Institute, Walnut Creek, California
Money Allocated: $704,400 (FY 1972 Funds)

Objectives: The objective of this program is to evaluate a variety of biochemical and behavioral parameters as related to contraceptive use in women who are members of the Kaiser Permanente Medical Care Program in Walnut Creek, California. The principal tool for accomplishing this objective is an automated multiphasic examination in which more than 17,000 women have been studied at least once. The long-range objective is to make longitudinal observations on various components of the automated multiphasic examination and to relate these to morbidity and mortality in the population.

Significance to Biomedical Research and Program of the Institute: This is a broad ranging program of surveillance of the possible effects of the use of various contraceptive methods with particular emphasis on the oral contraceptives. The particular value is the ability to monitor a large population in considerable depth and to be able to relate biochemical to clinical effects. It is relevant to the Center for Population Research program to monitor medical effects of oral contraceptives.

Proposed Course: Emphasis during the past year has been on obtaining repeat examinations on women who have been seen at least once in the program. Both cross-sectional and longitudinal analyses of the various components of the automated multiphasic examination are now underway. It is anticipated that a modification of the AML will be achieved on the basis of these analyses and that a limited laboratory program will be combined with a general monitoring and surveillance program to continue subsequently. Reports on the following subjects have been prepared and submitted for publication: blood pressure; red cell and white cell indices; carbohydrate metabolism; pulmonary function; depression and other behavioral effects; serum protein; latex titer; bacilluria; Papanicolau smear; thromboelastogram smoking and blood groups.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Physiological Mechanism Involved in Reactions to Implanted Devices in Animal Uteri
Contractor: Agricultural Research Service, U.S.D.A.
Money Allocated: $90,944 (FY 72 funds)

Objectives: The objectives of this program for the current year are to continue studies of 1) mechanisms regulating corpus luteum function in sheep and the interactions among such factors as endogenous and exogenous hormones, intrauterine devices, and a variety of drugs in their effects on the corpus luteum; and 2) the ultrastructure of the sheep endometrium, particularly in relation to changes which may be involved in luteolytic mechanisms; and to initiate studies of 3) the effects of progestational agents on circulating prolactin levels as a possible clue to effects of these agents on mammary tissue; 4) the spermicidal action and effects on uterine motility of progestins administered intravaginally; and 5) the possible toxicity of copper IUDs on developing sheep embryos.

Major Findings: A large amount of work has been performed, including experiments in each of the areas planned, but only preliminary data are available at this time. In many of the experiments, hormone determinations or data analyses were not complete at the time the most recent report was prepared, and in the case of studies of effects of copper IUDs on embryonic development, a number of pregnancies have been established with the IUD in contact with the chorionic membranes, but these pregnancies have not yet come to term. Thus, final results of this work will not be available for several months.

Significance to Biomedical Research and the Program of the Institute: Over the years this program has made significant contributions to knowledge of the varied effects of intrauterine devices and to more basic aspects of reproductive physiology. The program is now changing in direction but it is expected to continue to provide valuable data on the effects of progestational agents and endogenous hormones on reproductive function.

Proposed Course: It is expected that this program will probably be continued with a shift in emphasis away from the earlier studies of luteolytic mechanisms and the effects of IUDs and toward further work on effects of microsomal enzyme induction and synthetic progestins on reproductive function, the control of prolactin release, and the relationship between circulating hormone levels and endometrial ultrastructure.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : Chromosomal Breakage in Women Taking Oral Contraceptives
Contractor: AEC/Oak Ridge Associated Universities, Inc.
Money Allocated: $95,900 (FY 72 funds)

Objectives: The purposes of this project are to confirm and extend earlier studies which suggested that use of oral contraceptives leads to an increased frequency of chromosome breaks in cultured lymphocytes, and to determine whether the increased chromosome breaks in lymphocyte cultures are representative of chromosome damage in other somatic cells.

Major Findings: Lymphocytes have been cultured from 26 women taking oral contraceptives, 21 control women, and 10 control men; 50,000 cells in 587 cultures have been analyzed and analysis of an additional 178 cultures is in progress. The percentage of cells with one or more chromosome lesions is highest in cultures from women taking oral contraceptives (9.2%), intermediate in control women (6.5%), and lowest in control men (5.6%); the differences between women on oral contraceptives and each of the control groups are statistically significant ($P \leq 0.001$). Cells with two or more chromosome lesions occur more frequently in oral contraceptive users (15% of abnormal cells) than in each of the control groups (8%). The types of specific lesions and their ratios to total lesions are similar in cultures from women on oral contraceptives and control women. The frequency of cells with chromosome breaks varies widely among individual cultures in all three groups, and variability is higher in samples from both groups of women than in those from men; nevertheless, the percentage of cultures with more than 10% abnormal cells is strikingly higher in women on oral contraceptives. A higher incidence of breakage is also seen in cultures obtained in late spring and early summer in all three groups. Finally, a markedly higher incidence of abnormal chromosomes is seen in four women taking sequential type oral contraceptives than in those on combination medication, and the highest frequencies occur during the progestational phase of sequential medication. Studies of fibroblast cultures are also underway but the data will not be analyzed until this phase of the work is further advanced.

Significance to Biomedical Research and the Program of the Institute: Oral contraceptives are the most widely used method of fertility control, but there is very little information on their possible genetic effects. Chromosome breaks in peripheral leukocytes are induced by many agents including carcinogens and LSD; the significance of such breaks is not known but they probably reflect damage to other somatic cells and perhaps also to gametes. Although the full biological significance of chromosomal breakage is not understood, it is important to evaluate this effect on an agent to which large numbers of women are exposed.

Proposed Course: The studies originally proposed under this interagency agreement have now been completed, but the agreement may be renewed for additional

years to allow completion of the recently initiated studies of fibroblasts and study of larger numbers of women and additional women on sequential medication.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: An Epidemiologic Case-Control Study of Thromboembolism Related to the Use of Oral Contraceptives
Contractor: Johns Hopkins University - School of Hygiene & Public Health
Money Allocated: $69,399 (FY 1972 Funds)

Objectives: The purposes of this study are: 1) to investigate further the association between oral contraceptive use and thromboembolism in order either to substantiate or to define further the risks of developing these phenomena among users; 2) to provide a continuing surveillance of the risks of thromboembolic disease associated with the usage of oral contraceptives and to monitor adverse reactions to new formulations as they come into wider use; 3) to define further any possible predisposing factors that may increase the risk of developing thromboembolic disease in oral contraceptive users; 4) to determine if there is a difference in the relative risk of developing thromboembolic disease associated with different formulations of oral contracepitves, e.g., sequentials and non-sequentials; and to determine if a dose-response relationship occurs with increasing amounts of estrogen; and 5) to investigate further the reported proportional lesser incidence of thromboembolism in persons of blood group 0.

Major Findings: Two hundred and fifty cases with 3 matched controls per case have been collected and 100 cases and their controls have been analyzed. The preliminary results suggest a lower relative risk of thromboembolism than has been determined in previous studies. Data are insufficient to evaluate risk on the basis of estrogen dose of the formulations.

Significance to Biomedical Research and Program of the Institute: Several studies have now established an association between oral contraceptive usage and the development of thromboembolic disease. Questions as yet unanswered concern the relative risk of developing thromboembolic disease as related to usage of different formulations of oral contraceptives containing various amounts of estrogenic compound or a progesterone. In addition, it is still unclear which medical conditions or predisposing factors increase the risk of developing thromboembolic disease among oral contraceptive users. Studies supported by this contract should provide essential information to establish or rule out potential contraindications to the use of oral contraceptives. It should also help to determine which formulations of these agents present the greatest hazards to users. This study is important, well-designed, and is being carried out by an experienced group of investigators. The significance of the study is re-emphasized by recent articles questioning the association of oral contraceptives and thromboembolism.

Proposed Course: The research effort is planned to continue without significant change of protocol, except to reduce the number of participating hospitals from 60 to 30 or 40 of the most productive units. It is anticipated that the goal of 400-500 case totals will be reached at the end of this contract year.

Certain hospitals are being excluded because of making only small contributions to the patient totals. For logistic reasons efforts are now being focused on the Baltimore area. Three matched controls will continue to be collected for each case. Further reliability and validity checks will be carried out. The final analysis is proceeding and expected to be ready shortly after termination of case collection.

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Center for Population Research
Fertility Regulation Methods Evaluation Branch

Contract Title: Effect of Oral Contraceptive and Conjugated Estrogens on Renin Activity and Aldosterone
Contractor : Loma Linda University, Loma Linda, California
Money Allocated: $20,875 (FY 1972 Funds)

Objectives: This study has three principal goals. Firstly to study the biochemical effect of various estrogenic and progestogenic compounds on the renin-angiotensin-aldosterone system as well as on sodium metabolism of normotensive contraceptive users. Secondly, to study these effects in persons developing hypertension either in young women on the oral contraceptive pill or in menopausal women receiving conjugated estrogen replacement therapy (Premarin). Thirdly, to evaluate the efficacy of spironolactone to reduce estrogen induced elevated blood pressure.

Major Findings: Effort during the past year was largely concentrated on characterization of the renin-angiotensin-aldosterone system and fluid volume homeostasis as affected by estrogens and progestational agents in normotensive subjects. The experimental groups were as follows: eight subjects on ethynyl estradiol, ten on ethynyl estradiol plus aldactone, ten pre-menopausal and ten post-menopausal women on Premarin, ten subjects on ethynodiol diacetate and ten on diethylstilbesterol. Ethynyl estradiol raised plasma renin activity and increased the aldosterone excretion rate under basal conditions and caused (on an average) a greater response in these parameters to the stimulation of sodium restriction and/or standing. Plasma aldosterone levels (fasting and ambulatory samples) were slightly lower after one month on the medication, but there was no effect on suppressibility of plasma aldosterone with partial immersion in water. The combination of a spironolactone (Aldactone 25 mg daily) with the ethynyl estradiol caused a more pronounced increase in the renin activity values and aldosterone excretion rate (under basal conditions and during sodium restriction and/or standing) than did ethynyl estradiol alone. The plasma aldosterone levels on the average were also increased, but they suppressed normally with partial immersion. The conjugated estrogen, Premarin, in the younger age group and ethynodiol diacetate caused no apparent effect on renin activity. However, the average aldosterone excretion rate tended to be lower during a normal sodium intake and the basal plasma aldosterone levels tended to be lowered during the first month on the medication. In the older age (post-menopausal) group, Premarin did not appear to cause a decrease in aldosterone excretion or basal plasma aldosterone possibly because these were initially low to begin with. This will need to be examined further. Thus far, with the very limited data, stilbesterol seems to cause changes in renin activity and aldosterone levels similar to those observed from ethynyl estradiol.

Significance to Biomedical Research and Program of the Institute: The scientific value of the proposed work lies in its promise to clarify pathophysiological relationships between steroid metabolism, electrolyte balance and hypertension. This knowledge is relevant to the program of the Center in order to assess risk and consequences of contraceptive induced hypertension and possibly devise an approach to its therapy and/or prevention. Progress on the studies of normotensive steroid users appears to be satisfactory and should lead to important findings. The available description of results provides no information on the relationship between length of drug usage and time sequence in which abnormalities develop. This should be forthcoming from the final analysis of the data. It may lead to more refined hypotheses for further studies on contraceptive induced hypertension.

Proposed Course: It is estimated that the data analysis and manuscript preparation of the ongoing work on normotensive steroid users may extend for one or two months into the new contract period. After completion of this phase of the project, efforts will be concentrated toward studies of hypertensive subjects. After excluding renal vascular or other known causes of hypertension the following examinations will be performed: exchangeable sodium and renin substrate concentrations will be determined under basal conditions, plasma renin activity, urinary and plasma aldosterone levels under basal conditions and after stimulation or suppressive maneuvers will be assessed. Subsequent to this evaluation, Premarin will be discontinued and the patients followed to determine whether blood pressure returns to normal. If this is not the case it will be attempted to normalize blood pressure by spironolactone administration. After reaching a normotensive state the renin-angiotensin-aldosterone system will be reevaluated and the patients placed on another medication (diethylstilbesterol) to observe whether hypertension reappears and abnormalities in the renin-angiotensin-aldosterone system develop.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Oral Contraceptive Agents and Thromboembolic Complications
Contractor: Washington University School of Medicine, St. Louis, Mo.
Money Allocated:

Objectives: The objectives of this contract are: 1) to develop, improve and standardize a new diagnostic method of fibrinogen chromatography; 2) to demonstrate the clinical usefulness of fibrinogen chromatography as a screening procedure for assessing the risk of thromboembolic complications in patients on various types of contraceptive therapy; employ the method of fibrinogen chromatography in a clinical trial of an antithrombolic agent (prostaglandin, PEG_1).

Major Findings: Technical developments on the methodology of fibrinogen chromatography have been successful in standardizing and automating the procedure. It allows Dr. Fletcher to perform a large number of analyses in a reproduceable manner. The methodology remains complex and requires expertise and sophistication in protein chromatography. Consequently the prospects for a wider application outside Dr. Fletcher's laboratory are not very promising. Due to administrative difficulties the contemplated clinical trial with the Searle Company has not become operational, rather clinical studies were limited to patients somewhat non-systematically supplied by Dr. Burstein in St. Louis. Results of this work revealed that 20% of contraceptive users develop transient abnormalities in their fibrinogen chromatographs. In some patients frequent transitions from normal-abnormal-normal patterns are observed. A hypothesis has been advanced that these changes may represent episodic subclinical thrombosis followed by resolution and repeat thrombosis. Correlated with these biochemical changes are indefinite clinical symptoms suggestive of thrombosis such as pain, tenderness and swelling not necessarily limited to the lower extremities.

Significance to Biomedical Research and Program of the Institute: The development of a laboratory test capable to detect or predict intravascular coagulation is urgently needed in several fields of clinical and investigative medicine. It is of particular importance in contraceptive management, since unexpected thromboembolism appears to be a major well-documented side effect of contraceptive steroids.

Proposed Course: The major efforts during the next contract period will be directed toward performing a valid clinical trial with satisfactory experimental design. A collaboration with the Searle Company is contemplated. The company has developed a protocol for performing serial chromatographic and clinical examinations in 60 contraceptive users and controls. A second thrust for future work is towards developing criteria and methodology for diagnosing subclinical thrombosis. Plans are being developed to use an impedance plethysmograph for this purpose. The status of the prostaglandin subcontract with Dr. O'Brien is uncertain. A project site visit will be

conducted on May 18, 1972, to permit a better assessment of the direction of this program.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Metabolism and Metabolic Effects of Steroidal Oral Contraceptives
Contractor: Worcester Foundation for Experimental Biology
Money Allocated:

Objectives: The lines of research supported by this contract are aimed to: 1) define and identify metabolites of natural and synthetic estrogens and progestins; 2) measure the metabolic clearance, plasma half-life and rates of conversion of the various steroids.

Major Findings: 1) The metabolism of estradiol-17β has been studied in three women who have never taken oral contraceptive steroids and in seven women who have taken oral contraceptive steroids for a prolonged period of time. After administration of labelled estradiol, urine was collected for 96 hours and fractionated. The percentage of radioactivity in total urine and in various urinary fractions has been determined. Work is in progress to determine the ratio of eight metabolites (estrone, estradiol, estriol, 2-hydroxyesterone, 2-hydroxyestradiol, 16α-hydroxyesterone, 2-methoxyesterone and 2-hydroxyesterone 3-methyl ether) in urinary glucuronide and sulfate fractions. So far the fractionation was rather unrevealing and no significant differences in the peripheral metabolism of estradiol between pill-users and non-users were found.

2) Eight female rats were given single injections of double-labelled mestranol (M) or tritiated ethynylestradiol. Subsequently, the percentage of the administered labels (^{3}H and ^{14}C) were determined in bile urine and feces of each rat. Fractionation and isolation of biliary metabolites is in progress and some preliminary results are described for the work-up on one rat.

3) In the pharmacokinetic analysis the same patients were used as in the study of urinary metabolites of estradiol-17β. Single doses of tritium-labelled estradiol were administered to three non-pill users, three long-term contraceptive users taking 50 µg mestranol and to one female on 50 µg ethynyl estradiol. Constant infusions of tritium-labelled estradiol were administered to three chronic users of 50 µg ethynyl estradiol. The radioactivity in plasma was determined at various time intervals after injection. The concentrations of free estradiol in the plasma were expressed as fraction of administered dose of radioactivity per liter of plasma. From these data, volume of initial distribution (V_1) and metabolic clearance rates were calculated. Limited available results suggested that the three non-pill users and the three mestranol users did not differ either in their initial volume of estradiol distribution or in their metabolic clearance rate for estradiol. Chronic ethynyl estradiol ingestion increased the metabolic clearance rate of estradiol and its initial volume of distribution.

Significance to Biomedical Research and Program of the Institute: The proposed projects on metabolism and pharmacokinetics of natural and synthetic

estrogens in users and non-users of contraceptive steroids are highly relevant. They may contribute towards providing a scientific base for the medical and metabolic assessment of contraceptive therapy by means of anovulatory drugs.

Proposed Course: The proposed research for the next contract period does not differ significantly from that in the original proposal and workscope, except that the part dealing with the effects of oral contraceptive steroids on tryptophan metabolism has been eliminated. The following priorities for work during the next contract period are specified.

1. Urine from women ("Pill" and "Non-pill") who have received radioactive estrone will be worked up as described in the progress report for the estradiol experiments to assess any changes in the peripheral metabolism of this compound.
2. Isolation and characterization of ethynyl estradiol and mestranol metabolites from rats, guinea pigs and sheep will continue.
3. Urine from women injected with radioactive ethynyl estradiol and mestranol will be used for isolation and characterization of metabolites of these two synthetic estrogens.
4. The preparation of radioactive norethynodrel has begun and will be completed. This material will be used by dynamic studies outlined in Section II and for metabolite isolation work in women.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Studies of Dose-Response Relationships Between Low Dose Steroid Contraceptive Drugs and Plasma Gonadotropin Levels
Contractor: University of Rochester School of Medicine and Dentistry
Money Allocated:

Objectives: The general purpose of this proposal:to study systematically the plasma gonadotropin response to a number of combinations and dosages of these drugs with the expectation that one or more low-dose combinations can be found which consistently block ovulation with little or no effect on blood coagulation factors. Another specific aim of this proposal is to establish the lowest effective dose of several combinations of synthetic estrogens and progestogens which will reliably suppress the ovulatory surge in LH and FSH, and at the same time will be associated with either minimal or no measurable changes in blood coagulation factors II, VII, VIII and X, which are known to be elevated during oral contraceptive therapy. A secondary aim of this proposal is to identify which effective combinations have the least objectionable side effects of hyperestrogenism.

Major Findings: Four groups of women were investigated over a period of 5 treatment or menstrual cycles with appropriate control periods before and after drug treatments. The four experimental groups were: 1) women receiving a placebo; 2) women receiving 1.0 mg of megestrol alone; 3) women receiving 1.0 mg megestrol in combination with 50 mg ethynyl-estradiol and 4) women receiving 1.0 mg megestrol and 25 mg estradiol. Megestrol alone caused a complete suppression in the ovulatory surge of LH and FSH. The combination with the two dose levels of estrogen had no further effects. Consequently, no dose response date has been generated. None of the drug schedules had measurable effects on the coagulation parameters; side effects such as continuous spotting, irregular bleeding and abdominal cramps were pronounced.

Significance to Biomedical Research and Program of the Institute: A number of studies have indicated the possible importance of minimizing the dosage or adjusting the proportion of estrogen and progestin in presently available contraceptive formulations. It is likely that at least some of the undesirable side effects of these agents might be minimized by reducing the dose of drug to a level which is associated with adequate efficacy but minimal undesired effects. The study will give useful insight into this matter at least for certain drugs and may indicate the eventual utility of this particular type of approach as a more general evaluation procedure. From the results obtained so far, however, the combination of 1.0 mg megestrol with varying doses of estrogen appears to hold little promise for further investigation or practical use.

Proposed Course: A detailed protocol for future studies will be developed after completion of the megestrol studies. It is contemplated to investigate another progestogen, presumably norethindrone in combination with an estrogen. Based

on the negative experience gained during the past year, great care will be given to select a dose range wherein graded responses on FSH and LH will be observed.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: A Study of the Metabolism and Effects of Steroidal Oral Contraceptives
Contractor: Medical College of Georgia
Money Allocated: $39,590 (FY 72 funds)

Objectives: The purpose of the studies supported by this contract is to provide quantitative information on plasma half-life, metabolism and absorption of synthetic estrogens and progestational agents in women with normal menstrual function. The drugs to be investigated are mestranol and norethindrone.

Major Findings: Standardized procedures for isolating labeled norethindrone and mestranol from plasma and for correcting for procedural losses have been developed. Using these procedures and acute intravenous injections and 3-hour infusions of labeled contraceptive steroids, plasma half-lives of the parent drugs were found to be much shorter and metabolic clearance rates considerably greater than values obtained by others in studies of the disappearance of total radioactivity. A significant amount of radioactivity remains in plasma in a form other than the free drug, presumably as a conjugate. Methods have also been developed for analyzing free and conjugated steroids in urine, and it has been found that only small amounts of radioactivity appear in the urine over the first three days; the remainder may be excreted in feces, retained as conjugates in blood, or stored in adipose tissue.

Significance to Biomedical Research and the Program of the Institute: Detailed knowledge of the metabolism of contraceptive drugs is necessary to understand the basic pharmacology of these agents and their possible long term effects. Studies conducted under this contract will provide metabolic data on two of the most commonly used contraceptive steroids in human subjects.

Proposed Course: It is expected that this project will be continued for one or more additional years.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Biochemical and Physiological Evaluation of an Oral Chemical Contraceptive Agent
Contractor: Baylor College of Medicine
Money Allocated: $61,040 (FY 72 funds)

Objectives: The purpose of this program is to develop and use analytical methods of high resolution and high sensitivity for studying the absorption, metabolism and excretion of norethindrone. Packed and capillary column chromatography, gas chromatography and mass spectrometry techniques, with associated computer technology, are to be employed.

Major Findings: Work has been conducted on development of methods for isolating norethindrone and related steroids from blood in submicrogram amounts, detecting and estimating quantities of the drug, and separating steroids on capillary columns. Several methods for extracting tritiated norethindrone from plasma have been tested and a procedure has been developed which yields recoveries of 90% from plasma containing nanogram quantities of drug. The preparation of derivatives of 19-nor-testosterone, 17α-ethynyl testosterone, norethisterone and norethynodrel for electron capture detection has been investigated; reaction with heptafluorobutyric anhydride in benzene or isooctane and pyridine is satisfactory for microgram amounts of drug but less so when the sample size is reduced to a few nanograms. Picogram amounts of the HFB derivatives of norethindrone and norethynodrel can be detected by gas chromatographic separation and electron capture detection. Gas chromatography-mass spectrometry techniques with single ion detection were not satisfactory due to losses in the system. Tests of blood samples with the gas chromatography-electron capture method indicate that blood constituents will not interfere with detection of the drug. High resolution capillary column gas chromatography methods are being studied; methods of fabricating and coating capillary columns suitable for steroid separations have been developed and further testing is in progress. Preliminary studies with the plasma chromatograph suggest that this instrument may permit higher sensitivity of detection.

Significance to Biomedical Research and the Program of the Institute: Detailed knowledge of the metabolism of contraceptive drugs is necessary in order to understand the basic pharmacology of these agents, and high resolution, high sensitivity methods for detecting the drugs and their metabolites are required for these metabolic studies.

Proposed Course: This contract is expected to be renewed for one or more years in order to complete the methodological studies and apply the techniques to studies of drug metabolism in human subjects.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972

Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Alteration of Thrombogenic potential by Oral Contraceptive Steroids in Rhesus Monkeys
Contractor: Bionetics Research Laboratories
Money Allocated: $6,977 (FY 72 funds)

Objectives: The purpose of this project is to determine whether administration of contraceptive steroids to rhesus monkeys leads to changes which may be related to the thromboembolic side effects seen in some women using oral contraceptive drugs. A wide range of hematologic tests is being conducted as well as ultrastructural studies of platelets and endothelial cells, and blood flow in mesenteric vessels is observed visually and recorded cinematographically. The drugs being studied are mestranol alone and mestranol and norethynodrel in combination, at normal human dosage levels and at ten times this dosage.

Major Findings: This contract was initiated late in FY 1971, and the work is progressing well. However, since the protocol calls for a period of conditioning of the animals, two pre-treatment cycles of baseline data, and seven cycles of drug treatment, with complete necropsy examination at the completion of drug treatment, no final results are yet available.

Significance to Biomedical Research and Program of the Institute: Thromboembolic disease in users of oral contraceptives is the most serious documented side effect of these agents. Little is known of the pathophysiological mechanisms which lead to this complication. Studies in animals, and particularly subhuman primates, may clarify these mechanisms and provide leads to prevention of thromboembolic complications in women.

Proposed Course: This study is expected to be completed in the fall of 1972. No extension of the work to other areas is anticipated at this time.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972

Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Five-Year Prospective Study of Abnormal Cervical Cytology
Contractor: Temple University, Institute for Survey Research
Money Allocated: $550,000 (FY 1972 Funds for nine months)

Objectives: This is a five-year prospective study of the possible relationship between changes in cervical cytology and the use of various methods of contraception. Thirty thousand women will be recruited from 10 geographic locations and Papanicolau smears, contraceptive history and sociological and demographic information will be collected. After the first year of recruitment patients will be followed with annual Pap smears and current medical history. Papanicolau smears will be interpreted both by a central as well as the local cytological laboratories. The principal objective of the study is to determine whether there is any relationship between the use of steroid contraceptive drugs and changes in cervical dysplasia; in addition, any cases of breast cancer which are detected during the course of the study will also be evaluated. Other background information with regard to changes in contraceptive use and the general characteristics of the contraceptive-using population will also be analyzed.

Significance to Biomedical Research and Program of the Institute: It is clear that the steroid drugs used in oral contraceptive preparations have a stimulatory effect on the tissues of the uterine cervix as well as the breast. Whether these changes could result in a development of cancerous lesions remains to be determined. Since the etiology of genital system cancer is likely to be multifactorial, it is important that comprehensive and detailed studies of all factors including contraceptive use be collected simultaneously on the population at risk. The project is relevant to the mission of the Center for Population Research and its goal of assessing the medical effects and possible risks of contraceptive use and their correlations with other environmental factors.

Proposed Course: The study completed its planning and field logistics while awaiting clearance of the various study forms and instruments. This approval was received during Spring, 1972, and initial recruitment of the population will begin after May 1, 1972. It is anticipated that the cohort of approximately 32,000 patients will be able to be recruited during the course of one year.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Human Metabolism of Steroid Contraceptives
Contractor: University of Arizona
Money Allocated: $150,000 (FY 1972 Funds)

Objectives: The purpose of the program is to evaluate the metabolism of contraceptive steroids given in women who have had complications while on oral contraceptives and also who are receiving concurrent therapy with other drugs. Specifically, it will evaluate whether there are unique steroid metabolic profiles in women having had serious side effects of the oral contraceptives. In addition, it will determine the effects of both acute and chronic administration of other drugs in women who are on chronic courses of oral contraceptive agents.

Significance to Biomedical Research and Program of the Institute: This program represents a unique opportunity to determine whether women who have had complications on the oral contraceptives might have some unusual metabolic pattern which might serve as a basis for the clinical effects. In addition, since women are taking oral contraceptives for long periods of time, it is likely that they will be using other potent and effective drugs during the course of this treatment. The possibility of a significant interaction between the use of concurrently used drugs and steroid contraceptives should be evaluated. This program is relevant to the objectives of the Center for Population Research's goal of determining special risks following acute and chronic administration of steroid contraceptive drugs.

Proposed Course: The program has proceeded as anticipated correlating steroid and drug metabolism parameters in the population described. Continuation of the program to include other steroids and drugs is presently under consideration.

Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Study of Differential Effects of Oral Contraceptives on Carbohydrate Metabolism
Contractor: New York University - School of Medicine
Money Allocated: $20,000 (FY 1972 Funds)

Objectives: This study is designed to determine whether individual oral contraceptive formulations have unique effects on blood glucose, triglycerides, insulin and growth hormone in women who are obese, pre-diabetic or diabetic. Different preparations representing different dose levels of drugs will be studied in the same women to assess these changes.

Significance to Biomedical Research and Program of the Institute: It has been well established that oral contraceptive drugs cause decreased glucose tolerance and increase in circulating lipids. Whether these changes predispose, make worse or cause diabetes as well as arteriosclerotic cardiovascular disease is uncertain. It is not knownwhether women who are at risk of having diabetes mellitus are affected adversely by the use of these steroid drugs. The purpose of the program is to evaluate these potential risks in a population in special need of birth control. In addition, information of general value for contraceptive use in population would be made available.

Proposed Course: It has been difficult to recruit an adequate number of patients judged to be at high risk: obese, pre-diabetic or diabetic. There have also been questions raised about the accuracy and precision of the laboratory work being performed. Accordingly, an interim extension of the study for 3 months and the performance of a site visit to determine the likely success of recruitment as well as the accuracy of the laboratory work will be performed before deciding upon further continuation of the program.

NICHD ANNUAL REPORT
July 1, 1971 through June 30,1972

Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : Repository for Tissue Evaluation of Contraceptive Steroids
Contractor : Universities Associated for Research and Education in Pathology, Inc.
Money Allocated : $87,000 (FY 1972 Funds)

Objectives: The purposes of this contract are to establish at the Armed Forces Institute of Pathology a central repository for tissue specimens from animals, particularly beagle dogs, receiving hormonal contraceptive agents and to conduct pathological evaluation of the tissues. Contribution of tissue specimens will be solicited from pharmaceutical companies, the Food and Drug Administration laboratories and its contractors, contractors conducting relevant research projects for the Center for Population Research, and others. Standardized procedures will be used in processing specimens,and interpretation of pathology will involve consultative circulation through appropriate divisions of the Armed Forces Institute of Pathology.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are now required prior and subsequent to approval for clinical use. A major aspect of this testing is long term study in beagle dogs, a species which is unusually prone to development of breastnodules. Several promising contraceptive steroids have been removed from the market when breast nodules developed in beaglestreated with these compounds, but there is little information on the pathology of the beagle lesions or their possible relationship to human cancer. This contract was awarded to meet the need for central, expert and unbiased review of tissues obtained from various laboratories and processed with standardized methods; it is hoped that the nature of these lesions may thus be determined.

Proposed Course: It is expected that this contract will be renewed annually for a number of years.

NICHD ANNUAL REPORT
July 1, 1971 through June 30,1972

Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: A Comparative Study of Progestin Metabolism in Animals
Contractor: Pennsylvania State University
Money Allocated: $318,600 (FY 1972 Funds)

Objectives: This contract supports comparative studies of the metabolism and mechanism of action of progesterone and medroxyprogesterone acetate in monkeys, dogs, sheep, rats and mice. Investigations will be conducted in four areas: 1) metabolic clearance rates of the progestins and of sex steroid hormones, and binding capacities of steroid binding globulins, in acute and chronic studies with infusion of increasing dosages of progestin; 2) in vivo hepatic extraction of progestins and testosterone, effects of variation in bile flow, rate of steroid infusion, and rate of hepatic blood flow, with determinations of biliary metabolites and hepatic steroid metabolizing enzymes; 3) the effects of progestins on extra-hepatic steroid metabolism by determining extra-hepatic clearance rates, 5α-pregnane-3,20-dione levels in peripheral and hepatic venous blood, steroid content in a wide range of extra-hepatic tissues, and androgen-induced enzymes in kidney cell fractions; and 4) the effects of progestins on gonadotrophin secretion and reproductive function, hepatic drug metabolizing enzymes, mammary tissue pathology, and preputial glands. Radiolabeled progestins and metabolites of high specific activity will be synthesized for these studies and for distribution to other investigators.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are now required prior and subsequent to approval for clinical use, but interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot readily be extrapolated to man. Specifically, significant differences in absorption, metabolism, excretion and biologic potency may account for effects which may be unique to the species under evaluation but not relevant to man. Studies supported by this contract will contribute much needed comparative information on the metabolism and biological effects of progesterone and medroxyprogesterone acetate in five species. The investigations are expected to be extended to other synthetic progestins and to human subjects in subsequent years.

Proposed Course: It is expected that this contract will be renewed for two or more subsequent years in order to conduct similar studies of a number of additional progestational agents.

NICHD ANNUAL REPORT
July 1, 1971 through June 30,1972

Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: The Metabolism of a Derivative of 17α Acetoxyprogesterone in Beagles and in Rhesus Monkeys
Contractor: University of Colorado Medical Center
Money Allocated: $62,000 (FY 1972 Funds)

Objectives: This is an 18-month pilot study in which the metabolism of a derivative of 17α acetoxyprogesterone will be studied in dogs and monkeys. The number and relative quantities of urinary and biliary or fecal metabolites of orally administered radioactive progestin will be determined by classical chromatographic methods, and qualitative identification will be obtained by gas chromatography-mass spectrometry techniques. Metabolic profiles in the two species will be compared and species differences in metabolism of the administered progestin will be identified.

Significance to Biomedical Research and Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are now required prior and subsequent to approval for clinical use, but interpretation of the results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions can therefore not be readily extrapolated to man. This study and the work to be conducted in subsequent contract periods should clarify the metabolism of an important class of contraceptive steroids in two widely used test species and establish the degree of relationship between effects in these species and in human subjects.

Proposed Course: The present contract is for an 18-month pilot study. It is expected that this study will subsequently be extended to determination of plasma levels of parent compounds and metabolites, to other 17α acetoxyprogesterone derivatives, and to human subjects. Attempts will be made to identify metabolites unique to each species, and the effects of unique metabolites on the occurrence of breast nodules and on glucose tolerance will be investigated. Effects of the progestins on the renin-aldosterone-angiotensin system and interactions with other drugs will also be studied.

Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Development of Assay Methods for Circulating Polypeptide and Steroid Hormones in Beagle Dogs
Contractor: Cornell University College of Agriculture and Life Sciences
Money Allocated: $61,243 (FY 1972 Funds)

Objectives: This is an initial one-year contract for development of radioimmunoassay methods for LH, FSH, prolactin, estrone and estradiol and protein binding assays for measurement of progesterone and glucocorticoids in plasma samples from beagle dogs. Antisera available from other species will be tested for use in dog plasma, and other antisera will be developed if necessary. Protein binding assays now in use for other species will be adapted for studies in dogs.

Significance to Biomedical Research and Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are now required prior and subsequent to approval for clinical use, but interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions can therefore not readily be extrapolated to man. The present contract is designed to develop methods for use in subsequent studies of the effects of mestranol and norethindrone on endogenous hormones and a variety of other biological parameters in beagle dogs. The project is expected to contribute to understanding of the biological and pathological effects of two widely used contraceptive steroids in a species which is a required test animal for these drugs.

Proposed Course: When assay methods of appropriate specificity and sensitivity have been developed, the contract will be extended to determination of the levels of endogenous polypeptide and steroid hormones in the normal estrous cycle of the beagle. Thereafter, the effects of mestranol and norethindrone on hormone levels, liver and kidney function, hematological profiles, fertility and reproductive wastage will be investigated and detailed examinations will be carried out at necropsy to determine the effects of these contraceptive steroids on reproductive, endocrine and hepatic tissues.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Development of Radioimmunoassays for Norethindrone, Norgestrel, Ethynyl Estradiol and Mestranol
Contractor: Roswell Park Memorial Institute, Springville Laboratories
Money Allocated: $46,000 (FY 1972 Funds)

Objectives: The purpose of this contract is to develop radioimmunoassay methods for determining the levels of two progestins (norethindrone and norgestrel) and two estrogens (ethynyl estradiol and mestranol) in human blood and urine and in blood, urine and tissues of monkeys and beagle dogs. The assays will subsequently be used in studies of the absorption, metabolism, excretion, and biological effects of these contraceptive steroids in these three species.

Significance to Biomedical Research and Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are now required prior and subsequent to approval for clinical use, but interpretation of the results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore can not be readily extrapolated to man. Specifically, significant differences in absorption, metabolism, excretion and biologic potency may account for effects which may be unique to the species under evaluation but not relevant to man. The present study is a necessary preliminary to subsequent investigations which will provide information on the suitability of tests in beagles and monkeys for predicting the effects of four of the commonly used contraceptive steroids in man.

Proposed Course: When radioimmunoassays of appropriate selectivity and sensitivity have been developed, it is expected that this contract will be extended to studies of absorption, metabolism, urinary and biliary excretion of these steroids and their effects on blood coagulation, the fibrinolytic system, liver and kidney function, mammary glands, fertility and reproductive wastage, and pathology as determined at autopsy.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Study of the Comparative Pharmacology of Steroid Contraceptive Drugs
Contractor: Institute of Pharmacological Research "Mario Negri"
Money Allocated: $172,068 (FY 1972 Funds)

Objectives: This 18-month contract supports the first half of a 3-year program of studies in four major areas: 1) The effects of steroid contraceptive drugs on chemical mediators in the brain and in target organs; 2) effects on immunological responsiveness and the invasiveness of cancer; 3) effects on lipid metabolism and lipolysis in adipose tissue; and 4) the interaction of steroid contraceptive agents with other drugs. In all of these investigations, data will be correlated with levels of the steroid contraceptive agents in blood and tissue and with antifertility effects. Studies will be conducted in mice, rats, guinea pigs and rabbits. The animals will receive one of three drug combinations administered orally (norethynodrel plus mestranol, norethindrone plus mestranol, and lynestrenol plus mestranol), and in some experiments additional animals will receive the individual steroids alone. A range of dosages will be used in both acute and chronic studies, and dose-response effects will be determined.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are now required prior and subsequent to approval for clinical use. In line with standard toxicologic practice, drugs are administered in arbitrary multiples of the likely or proven therapeutic dose in humans, and the biologic endpoints to be measured are intended to represent the effect to be considered in humans. However, interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot readily be extrapolated to man. This contract supports a wide range of dose-response studies of the effects of contraceptive steroids in laboratory animals; much of the work is imaginative and is likely to yield valuable new information on the effects of these widely used drugs. The studies may subsequently be extended to larger animals and to humans.

Proposed Course: It is expected that the present contract will be renewed for an additional 18 months to complete the proposed work. If results in animal studies warrent, the studies of drug interactions may be extended to human subjects during the present or renewal contract period.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Peripheral Metabolic Effects of the Ethynyl Estrogens in Subhuman Primates and Canines as Compared to Man.
Contractor: Southwest Foundation for Research and Education
Money Allocated: $88,742 (FY 1972 Funds)

Objectives: Studies supported by this contract will determine the effects of two dosage levels of ethynyl estradiol and mestranol, alone and in combination with norethindrone, megestrol and norgestrel, on a range of metabolic functions in baboons and beagles and compare the results with similar data being obtained for human subjects under support from other sources. The effects of the steroids on glucose tolerance, plasma lipoproteins, and plasma cortisol, testosterone and androstenedione will be determined in both species, and in addition, effects on serum bilirubin, fibrinogen, alkaline phosphatase, SGOT, tryptophan, ceruloplasmin, thyroxine, and steroid-binding protein will be determined in the baboons.

Significance to Biomedical Research and Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are now required prior and subsequent to approval of these drugs for clinical use. In line with standard toxicologic practice, drugs are administered in arbitrary multiples of the likely or proven therapeutic dose in humans and the biologic endpoints measured are intended to represent the effect to be considered in humans. However, interpretation of the results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and, as a result, conclusions cannot readily be extrapolated to man. This study is expected to provide data on the effects of two dosages of the two estrogens most commonly used in contraceptive formulations, alone and in combination with three of the most commonly used progestins, in one of the required test animals (the beagle dog) and in the baboon which may prove to be more similar to man.

Proposed Course: It is anticipated that this contract will be renewed for two subsequent years in order to complete the proposed studies.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Pharmaco-Pathologic Effects of a 17α -Hydroxyprogesterone Derivative in Beagle Bitches.
Contractor: University of Rochester, School of Medicine and Dentistry
Money Allocated: $144,086 (FY 1972 Funds)

Objectives: This contract is to support studies of the effects of medroxy-progesterone acetate (MPA) on uterine and mammary gland structure and function in beagles in an attempt to explain the unusual sensitivity of this species to derivatives of 17α -hydroxyprogesterone. The minimum ovulation-suppressing dose of MPA will be determined, and the effects of this dosage and doses twice and half this amount on morphological, histochemical and biochemical characteristics of mammary glands and uteri will be investigated. Glands and uteri will be removed for study at intervals of 3-4 months for a period of two years, and complete necropsy examinations will be conducted after 12 and 24 months of treatment.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are now required prior and subsequent to approval for clinical use, but interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot readily be extrapolated to man. Long-term studies in beagle dogs are part of the required testing, and several promising contraceptive agents have been removed from the market when mammary nodules occurred in the beagles, a species which is unusually prone to development of breast lesions. Work under this contract should help clarify the nature of the beagle nodules and their possible relationship to breast cancer in human subjects.

Proposed Course: It is expected that this contract will be renewed for an additional year or two to complete these studies.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Biliary and Urinary Excretion, Enterohepatic Circulation, and Hepatic Effects of Contraceptive Steroids
Contractor: Health Research, Inc., Roswell Park Division
Money Allocated: $55,000 (FY 1972 Funds)

Objectives: The metabolites of six contraceptive steroids, including representatives of the 17α acetoxyprogesterone series and also of the 19-nor steroids, will be studied in baboons and in human subjects by injecting tracer amounts of radiolabeled steroids and determining urinary and biliary excretion of radioactivity and enterohepatic circulation and isolating and identifying urinary and biliary metabolites. In baboons, the effects of pharmacological doses of the steroids on biliary excretion, enterohepatic circulation, liver function, and damage to bile canaliculi and hepatic cells will also be determined.

Significance to Biomedical Research and Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are now required prior and subsequent to approval for clinical use, but interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions may not be readily extrapolated to man. Specifically, significant differences in absorption, metabolism, excretion and biologic potency may account for effects which may be unique to the species under evaluation but not relevant to man. Other species may be more suitable than those presently used for testing these drugs, and the baboon, which is phylogenetically closer to man, may prove to be an excellent choice. This contract is one of several initiated this year to study the metabolism and effects of contraceptive steroids in the baboon and determine its suitability as a test animal for these drugs.

Proposed Course: It is expected that this contract will be renewed for two subsequent years and that studies of six contraceptive steroids will be conducted each year.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Metabolism of Contraceptive Synthetic Estrogens
Contractor: Michael Reese Hospital and Medical Center
Money Allocated: $34,319 (FY 1972 Funds)

Objectives: This contract is to support studies of the metabolism of ethynyl estradiol and mestranol in baboons. Tracer doses of radiolabeled steroid will be administered during the proliferative phase and again during the luteal phase of the estrous cycle, and blood and urine samples will be obtained for metabolic studies. After an interval without medication, the animals will be given a combination type steroid contraceptive orally for two to three consecutive cycles, after which the tracer studies will be repeated. The disappearance of radioactivity and of the administered drug from blood will be measured and metabolic clearance rates and plasma half-lives will be calculated. Urinary metabolites will be isolated by chromatographic methods and hydrolysis of conjugates and identified by comparison with reference standards or by physical-chemical methods.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are now required prior and subsequent to approval for clinical use, but interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions can therefore not be readily extrapolated to man. Specifically, significant differences in absorption, metabolism, excretion and biologic potency may account for effects which may be unique to the species under evaluation but not relevant to man. Other species may be more suitable than those presently used for testing these drugs, and the baboon, which is phylogenetically close to man, may prove to be an excellent choice. This contract is one of several initiated this year to study the metabolism and effects of contraceptive steroids in the baboon and determine its suitability as a test animal for these drugs.

Proposed Course: It is expected that this contract will be renewed for two additional years to complete the proposed studies.

NICHD ANNUAL REPORT
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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: The Baboon: An Animal Model for the Study of Contraceptive Steroids
Contractor: New York Hospital - Cornell Medical Center
Money Allocated: $36,107 (FY 1972 Funds)

Objectives: The purposes of this contract are to develop a modified Thomas duodenal cannula for implantation in the baboon and to use baboons with implanted cannulas in studies of the effects of contraceptive steroids on biliary function. Several modifications in design of the cannula will be tested as possible improvements over a preliminary design which has already been implanted. A contraceptive steroid, probably chlormadinone acetate, will be administered over a wide dosage range to baboons with cannulas in place, and effects on hepatic excretory function, including bile salt metabolism and pool size, will be investigated. Tests of blood sugar, blood coagulation, serum lipoprotein patterns and liver function will also be performed. Some of the animals with implanted cannulas are expected to be used in collaborative studies with other investigators, particularly in investigations of the metabolism of contraceptive steroids.

Significance to Biomedical Research and the Program of the Institute: Extensive chronic studies of the therapeutic and potentially toxic effects of the contraceptive steroids in various animal species are now required prior and subsequent to approval for clinical use, but interpretation of results is often uncertain because the pharmacology and biological effects of these drugs may vary considerably among species and conclusions therefore cannot readily be extrapolated to man. Other species may be more suitable than those presently used for testing these drugs, and the baboon, which is phylogenetically close to man, may prove to be an excellent choice. This contract is one of several initiated this year to study the metabolism and effects of the contraceptive steroids in the baboon and determine its suitability as a test animal for these drugs. The studies supported by this contract should provide data on biliary effects of the steroids and also facilitate studies of their metabolism by other investigators.

Proposed Course: The present contract may be renewed for one or more subsequent years for studies of effects of other contraceptive steroids on biliary function.

NICHD ANNUAL REPORT
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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Immunopathologic Consequences of Vasectomy
Contractor: St. Louis University
Money Allocated: $113,754 (FY 1972 Funds)

Objectives: This contract provides for a two-year study of the effects of vasectomy in 500-1000 human subjects and in experimental animals. Sera will be monitored for antibodies to sperm; immunoglobulin classes of the antibodies, their cross-reactivity with other tissues and their ability to activate complement components will be determined; and leukocytes will be assayed for cell-mediated hypersensitivity. The patients will be followed, particularly for symptoms of a possible autoimmune response to sperm. Vasectomized animals will be autopsied at various intervals and pathological lesions will be sought in the testis and other organs, and attempts will be made to induce similar lesions by passive and adoptive transfer. The relationship between appearance of antisperm antibodies, degenerative changes in the testis, and sperm granuloma formation will be examined in vasectomized animals, and the role of an immune component in the sperm granuloma reaction to α-chlorhydrin will be studied in rats with induced tolerance to sperm.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possiblity that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated this year to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies under this contract should provide valuable new information on autoimmune reactions to vasectomy and the pathogenetic mechanisms which may be involved.

Proposed Course: This two-year contract was initiated at the end of FY 1972. Plans for additional years will depend on the results obtained in the currently supported studies.

NICHD ANNUAL REPORT
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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Immunologic and Morphologic Consequences of Vasectomy
Contractor: University of Missouri Medical Center
Money Allocated: $86,378 (FY 1972 Funds)

Objectives: The purpose of this program is to determine the morphological and functional changes subsequent to vasectomy, the role of the immune response in the development of these changes, and the possiblity of protection against immunologically mediated injury by certain immunoglobulin classes. Studies will be conducted in histocompatible strain 13 guinea pigs and in human patients. In the first year, tests of circulating antisperm antibody levels and their immunoglobulin classes, immediate and delayed hypersensitivity, macrophage migration inhibition, fertility, and pathologic and immunopathologic effects on reproductive and other tissues will be conducted in immunized, vasectomized and vasoligated guinea pigs. Studies in human subjects will include determination of antisperm antibody levels, immunoglobulin classes, and delayed hypersensitivity before and at intervals after vasectomy.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated this year to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies supported by this contract should contribute significantly to defining immunological mechanisms in experimental allergic aspermatogenesis and following vasectomy, and provide for correlation of data between the guinea pig model and vasectomized patients. Studies planned for subsequent years should further clarify immunopathogenetic mechanisms in transfer experiments and test the protective capacity of passive immunization against the immunologic consequences of vasectomy.

Proposed Course: It is expected that this contract will be renewed for one or more additional years to support continuation of these and related studies.

Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Immunologic Studies on Steroid-Producing Cells
Contractor: State University of New York at Buffalo
Money Allocated: $80,000 (FY 1972 Funds)

Objectives: This 2-year contract supports an immunologic study of steroid producing cells of the testis and their response to vasectomy. Sera from vasectomy patients will be tested for circulating antibodies to Leydig cells, adrenal and ovarian cells, using as controls sera from patients with Addison's disease which contain antibodies to these tissues. The antigens of the interstitial tissue of human and animal testes will be characterized by immunochemical and immunoenzyme methods. Experimental animals will be immunized with interstitial tissue and with steroid-protein conjugates or vasectomized for studies of antibodies and cell-mediated immunity, hormone levels, pathological examinations of the testes, and binding of gamma globulins to Leydig cells.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated this year to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. The studies under this contract should provide important information on possible autoimmune effects of vasectomy on Leydig cell function.

Proposed Course: This 2-year contract was initiated late in FY1972. Plans for additional years of support will depend on the results obtained in the present studies.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Vasectomy and Autoimmune Disease
Contractor: Memorial Hospital, Worcester, Massachusetts
Money Allocated: $39,650 (FY 1972 Funds)

Objectives: This contract supports studies of the immune sequelae of autoimmune aspermatogenesis and vasectomy in mice. Immunoglobulin profiles and cellular immune responses will be determined with a variety of techniques, evidence for autoimmune phenomena other than aspermatogenesis will be sought by such tests as antithyroid antibody levels and effects on LE preparations, and histopathologic studies will be conducted at autopsy with special attention to evidence for arthritis, endocrine and particularly adrenal tumors, and immune-complex glomerulonephritis.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated this year to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies under this contract provide for comparison between the effects of experimentally-induced aspermatogenesis and vasectomy and also should contribute important information on possible pathologic effects of vasectomy.

Proposed Course: This contract was initiated at the end of FY 1972 and plans for subsequent years have not yet been developed.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Ultrastructural and Immunochemical Studies of Sperm Antigens Involved in the Autoimmune Response in Vasectomized Mammals
Contractor: University of Miami
Money Allocated: $51,451 (FY 1972 Funds)

Objectives: The purposes of this contract are to study the effects of antisperm autoantibodies on sperm fertility, the localization of these antibodies in sperm and testicular tissue, and the role of permeability of the seminiferous tubule barrier in the development of an immune response to sperm. The effects of antisperm antibodies from immunized and vasectomized animals on sperm hyaluronidase activity and ability to disperse cumulus cells will be evaluated and the effects of vasectomy and immunoglobulins from vasectomized animals on sperm fertility will be tested in artificial insemination experiments. The localization of antisperm antibodies in sections of testicular tissue and in epididymal and ejaculated spermatozoa will be studied using light and electron microscopy and various antibody labelling techniques, and the permeability of the seminiferous tubule barrier to immunoglobulins and tracer molecules will be evaluated in immunized and vasectomized animals.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possiblity that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated this year to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies supported by this contract should help elucidate the mechanism of an autoimmune response to vasectomy and also contribute information on the possibility of subsequent restoration of fertility.

Proposed Course: It is expected that this contract will be renewed for two additional years to complete these and related studies.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: An Immunogenetic Analysis of Sperm Autoantigens in the Mouse
Contractor: Cornell University Medical College
Money Allocated: $89,653 (FY 1972 Funds)

Objectives: Under this contract the investigators will study sperm surface antigens directed by genes at the T locus in the mouse and determine whether these surface antigens can act as autoantigens and whether they are represented on cells of non-testicular tissues. Experiments will be conducted to determine the occurrence of autoantibodies to these antigens following vasectomy and to identify the classes of antigens to which the antibodies correspond. The production of antibodies will be monitored using a sperm cytotoxicity test developed by these investigators.

Significnace to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated this year to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies under this contract are expected to lead to interesting and highly significant findings on the nature of gene products on the sperm surface which may act as autoantigens under such conditions as vasectomy.

Proposed Course: It is expected that this contract will be renewed for one or more additional years to complete these and related studies.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Sperm Immunology
Contractor: Netherlands Cancer Institute
Money Allocated: $55,450 (FY 1972 Funds)

Objectives: This two-year contract supports studies in three areas. Sera from naturally infertile men which contain antibodies to spermatozoa will be used to detect autoantigens in sperm fractions, and immunochemical methods will be used to isolate and characterize the antigens. Sperm agglutinins in serum and seminal plasma of naturally infertile and vasectomized men will be characterized with respect to immunoglobulin class and the classes examined for specificity to certain parts of spermatozoa and association with various clinical types of infertility. A variety of modified and new techniques will be developed to measure antisperm antibodies, immunoglobulin classes, and cellular immunity against sperm.

Significance to Biomedical Research and the Program of the Institute: Vasectom for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated this year to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies under this contract are expected to contribute significant practical improvements in assay methods for antibodies and cellular immunity to sperm. If defined antigens can be isolated, a radioimmunoassay for sperm antibodies would be possible and new contraceptive approaches may also be developed. The characterization of immunoglobulin classes of sperm agglutinins is of fundamental interest and also may have practical application in predicting the efficacy of vas reanastomosis after vasectomy.

Proposed Course: The present two-year contract was initiated late in FY 1972; plans for possible additional years of support will depend on the results obtained in the present studies.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Effects of Vasectomy on Testes, Epididymis and Vas Deferens
Contractor: University of Virginia School of Medicine
Money Allocated: $104,070 (FY 1972 Funds)

Objectives: This contract supports two separate but related projects by two investigators in the same department. One study will examine the function of smooth muscle in the vas deferens and changes which may occur as a result of vasectomy; experiments will be conducted to measure mechanical properties of the vas, sperm transport, and histological changes in muscularis and connective tissue in animals and human subjects and the effects of vasectomy, age, hormones, drugs, and sexual activity on these parameters. The other study will utilize micropuncture techniques to study intraluminal pressure, sperm morphology, and sodium-potassium levels in the seminiferous tubules of normal,vasectomized and vasoligated rats.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposes is gaining increasing acceptance throughout the world, but there is a possibility that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated this year to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. Studies under this contract will provide new and much needed information on the physiological effects of vasectomy on organs of the male reproductive tract.

Proposed Course: This contract is expected to be renewed for an additional year to complete the studies proposed.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Endocrine Changes in Vasectomized Men
Contractor: University of Texas Medical School at Houston
Money Allocated: $200,000 (FY 1972 Funds)

Objectives: The purpose of this program is to conduct a prospective study of endocrine changes in men undergoing bilateral vasectomy. Plasma levels of testosterone, estradiol, 20 α-dihydroprogesterone, FSH, and LH will be determined in approximately 200 men prior to vasectomy and at 1 and 6 weeks and 3, 6, 12, and 24 months after vasectomy.

Significance to Biomedical Research and the Program of the Institute: Vasectomy for contraceptive purposed is gaining increasing acceptance throughout the world, but there is a possiblity that complex physiological and immunological effects may result from blockage of the excurrent ducts or extravasation of sperm outside normal channels. This contract is one of a number initiated this year to define the long-term consequences of vasectomy, the mechanisms involved in development of these alterations, and their relation to the possible occurrence of systemic complications. No data are presently available on the endocrine effects of vasectomy and such effects would not necessarily be expressed somatically or behaviorally. Accurate data on levels of testosterone, estradiol FSH and LH are therefore required, and 20 α-dihydroprogesterone levels may be indicative of testicular damage.

Proposed Course: This two-year contract was initiated late in FY 1972; plans for possible renewal for subsequent years will depend on the results obtained in the present studies.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Effect of Oral Contraceptive Steroids on Calcium, Phosphorus, Magnesium and Zinc Metabolism, with Emphasis on Bone Mineralization.
Contractor: Veterans Administration Hospital, Washington, D. C.
Money Allocated: $22,668 (FY 1972 funds)

Objectives: This proposal is designed to study the effect of steroidal contraceptive agents on mineral metabolism. The influence of a progestin, an estrogen, and a combination of these hormones on calcium, phosphorus, magnesium and zinc metabolism will be investigated. Particular attention will be focused on bone metabolism.

Significance to Biomedical Research and Program of the Institute: The effect of anovulatory steroids on calcium, phosphorus, magnesium and zinc has not been established in a definitive manner. All of these mineral nutrients are important in the continuous process of bone formation and resorption. No known calcium balance studies have been carried out in human subjects receiving the oral contraceptives. There is conflicting evidence in the literature regarding the effect of anovulatory steroids on serum calcium, phosphorus, magnesium and zinc. Some, but not other investigators, have been decreases in serum calcium, phosphorus, and magnesium during steroid ingestion; only the decreases of serum zinc appears well established. None of available information allows a conclusion as to the effect of steroids on the nutrition requirement for these minerals. It can be expected that the proposed study will show whether or not the contraceptive steroids produce an increased requirement, as expressed by negative balance. Particular emphasis is to be placed on calcium which will be studied at three dietary levels. The duration of the experiment is enough to allow for adaptation of the animals to low or high calcium takes, so that a valid interpretation can be made. It is important to assess the effect of the anovulatory agents on mineral metabolism since their prolonged use at a critical time (pre-pregnancy and pre-susceptibility to osteoporosis) may be detrimental to bone integrity.

Proposed Course: One hundred and twenty female rats will be equally allotted by weight into three principal groups receiving different amounts of calcium: A. Control diet (0.6% calcium), B. Optimal calcium diet (1.2%), C. Inadequate calcium diet (0.2%). These three groups of rats will simulate populations receiving a normal, high (such as during lactation), and low calcium intake. The three groups will be divided into four sub-groups with equal numbers of animals and will be fed the hormone(s) as follows: (i) no hormone, (ii) norethindrone, a progestin, (iii) mestranol, an estrogen, (iv) norethindrone and mestranol simulating a combination oral contraceptive agent. The hormones will be fed in the diet for 120 days in amounts proportional to the body weight dosage used with human subjects. During the study two five-day metabolic balance studies will be conducted to determine the retention of calcium, phosphorus, magnesium and zinc. At the termination of the investigation the

concentration of these nutrients in serum, femur and liver will be determined. Bone length, density, cortical thickness, crystallization and histology will serve as additional parameters.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Interaction of the Contraceptive Steroids and Vitamin K Absorption and Utilization
Contractor: Southern Illinois University, Edwardsville, Ill.
Money Allocated: $33,115 (FY 1972 Funds)

Objectives: Thromboembolic accidents have been reported to occur at a significantly higher rate in women on oral contraceptives than they do in control subjects. Cause of this phenomenon is not well understood. It has been postulated that the contraceptive steroids may substitute for vitamin K or vice versa, or they may influence the gastrointestinal absorption of vitamin K. The objectives of this proposal are to: determine whether the molecular configuration of a particular estrogen or progestogen can be related to its vitamin K potentiating, or substituting effects, or its ability to facilitate vitamin K absorption.

Significance to Biomedical Research and Program of the Institute: The studies outlined hold considerable promise of providing basic information concerning the significance and mechanism of an apparent interaction between oral contraceptive steroids and vitamin K. In view of the possible implication of these effects in the pathogenesis of thromboembolic disease, the project is clearly relevant to the objectives of the program.

Proposed Course: The first phase of the study would be concerned with investigating the mechanism whereby estrogenic steroids have been reported to substitute for vitamin K in the maintenance of normal coagulability. Several approaches will be employed: (1) the relative rates of vitamin K depletion will be studied in normal female, male and castrate rats given either an estrogen or progestogen, both separately and in standard anovulatory combinations to establish the relative contribution of these several factors to the coagulation picture; (2) the estrogenic effects of vitamin K depletion will be studied in the normal and castrate females and the potentiation of testosterone on vitamin K deficiency or inhibition measured; (3) a wide variety of estrogens and progestogens will be assayed with the objective of evaluating chemical structural requirements for both vitamin K activity and estrogenic effects. It is hoped that the results of these studies to be conducted during the first contract period will provide the basis and guidance for more specific enzymatic investigations. Moreover, the nutritionally oriented studies would provide information as to whether or not the steroids have an influence on the gastrointestinal absorption of vitamin K. If evidence is apparent of such an effect, studies will be conducted to evaluate its significance through the use of radioactively labelled vitamin K in rats with cannulated thoracic lymph ducts. Following the completion of these phases of the study, enzymatic experiments would be conducted to determine how and where the steroids fit into the vitamin K metabolism and how vitamin K can substitute for estrogen.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Biochemical Mechanisms of Contraceptive Steroid-Induced Abnormalities in Absorption, Metabolism and Function of Vitamin K.
Contractor: University of Nebraska College of Medicine
Money Allocated: $48,507 (FY 1972 funds)

Objectives: The purpose of this project is to elucidate the mechanism of estrogen-induced changes in the requirement for vitamin K-dependent clotting proteins, and the decrease in sensitivity to oral anticoagulants. Specific objectives are to: (1) Determine the effect of estrogen and androgen on the quantitative response of deficient rats to vitamin K, (2) Characterize the effect of estrogen on oral anticoagulation in the rat, and (3) Ascertain the localization of vitamin K and the activity of vitamin K metabolizing enzymes in the liver of estrogen-treated animals.

Significance to Biomedical Research and Program of the Institute: The most well documented serious non-contraceptive effects of the anovulatory steroids is the higher incidence of thromboembolic disease, resulting from such treatment. Vitamin K has been implicated in this syndrome more than any other nutrient. In general, there appears to be a positive interaction between vitamin K and the steroids which may be the result of "K"-like activity from the steroid or the steroid increasing the efficiency of K utilization. This study proposes to investigate this interaction by examining the effect of the steroid on K absorption, metabolism and utilization. The procedures outlined appear excellent for most of these tasks. Administration of the labelled and cold vitamin by intracardiac or tail vein injection do obviate the problems of absorption. Isolation of appropriate subcellular fractions should provide data to indicate if the steroid effect is on the receptor site. Concentration on the vitamin K metabolizing system should tell if activation is the principle point of attack.

Proposed Course: The scope of the project involves three lines of investigation. In the first part of the study, the requirement for vitamin K under various regimes of androgen and estrogen administration will be determined. In these studies, K-deficient rats with and without steroids will be given various levels of K in the diet. Blood samples will be assayed for K and for clotting factors such as prothrombin. The level of K in the liver will also be determined. In the second line of investigation, the effect of androgen and estrogen on retention and distribution of vitamin K in subcellular fractions of liver will be assessed. The influence of steroids on the enzymes responsible for the interconversion of vitamin K and its 2, 3 epoxide will be characterized. For the kinetic analysis of K metabolism under the influence of hormones H^3 and C^{14} labelled phylloquinone will be employed. A third line of work will attempt to characterize the mechanism of relative resistance to oral anticoagulants observed in pregnancy and under the influence of oral contraceptives. On the basis of his previous studies the applicant suggests

that genetic resistance to anticoagulants may be due to an alteration in a vitamin K metabolizing system which is normally sensitive to warfarin and responsible for the anticoagulant effect. The experimental approach used to examine the warfarin resistant trait will be applied to the relative resistance to warfarin elicited in pregnancy and the estrogen-treated animal.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Investigation of a Possible Interaction of Contraceptive Steroids with Vitamin E and Polyunsaturated Fatty Acids
Contractor: University of California - Los Angeles, California
Money Allocated: $38,788 (FY 1972 funds)

Objectives: It has been observed that in the rat the effects of administering oral contraceptive compounds resemble those resulting from vitamin-E deficiency. These include interference with reproduction, changes in lipoprotein distribution, and a decrease in polyunsaturated fatty acids in various tissues. The possibility exists that these anovulatory drugs enhance the requirement for vitamin E. Therefore, this investigation is designed to study the possible interrelationships between oral contraceptives and vitamin E. It is possible that oral contraceptive treatment results in situations where increased vitamin E and possibly increased polyunsaturated fatty acids are required for some essential physiological processes.

Significance to Biomedical Research and Program of the Institute: The project addresses itself to the problem whether oral contraceptives affect the vitamin E status of young women. This is relevant to the objectives of this program.

Proposed Course: It is proposed to administer the oral contraceptive drug Enovid E, to adult female rats kept on diets either adequate or deficient in essential fatty acids, with or without dietary tocopherol or with an antioxidant BHT (butylated hydroxytoluene) since in some investigations the antioxidant, BHT, has been shown to alleviate some vitamin E deficiency symptoms. The possible effect of tocopherol repletion in vitamin E-deficient animals given the oral contraceptive drug will be examined as well. At the end of the experimental period, animals will be killed and cholesterol levels and fatty acid patterns of lipid fraction of various tissues will be determined along with alpha/beta lipoprotein ratios, serum tocopherol levels and the susceptibility of erythrocytes to hemolysis, a test which has been used as an indication of vitamin E status.

In addition, a comparative survey of vitamin E status of young women given oral contraceptive drugs for various lengths of time, with untreated young women serving as controls, will be done. This will involve determinations of plasma tocopherol levels, plasma cholesterol and alpha/beta lipoprotein ratios.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Relationships of Contraceptive Steroids to Nutritive State
Contractor: Tulane University
Money Allocated: $110,532 (FY 1972 funds)

Objectives: This project is based upon the hypothesis, that since contraceptive steroids produce physiologically a "pseudo pregnancy" state, nutritional complications of pregnancy may develop in persons using these steroids. The general purpose of this proposal is to examine the nutritional status of a number of individuals, who are potentially at high risk nutritionally and attending a family planning clinic. Some will be taking contraceptive steroids and others will serve as controls. It is felt that if contraceptive steroids create nutritional stresses, these can be most easily identified by studying women who received CS through family planning clinics. The specific objectives of this project are to examine the nutritional status of 50 new and 100 long-time users of oral contraceptives who are potentially a high risk nutritionally. Results shall be compared with two appropriately matched control groups who use other methods of contraception.

Significance to Biomedical Research and Program of the Institute: This project promises to provide detailed information on the broad nutritional profile of young women beginning contraceptive therapy immediately after pregnancy and of long time contraceptive users. The characteristics of the population to be studied offers several unique advantages: Past experience indicates a high prevalence of anemia, marginal or low serum vitamin A levels and possible folic acid deficiency. The experimental design of the project appears scientifically sound and clearly elaborated. It should provide a broad spectrum of data which characterize the influence of contraceptive steroids on the nutritional health status in a population from a low socioeconomic level.

Proposed Course: The basic design of this proposal is to determine the nutritional health status of women in each of four groups. The first two groups, of 50 women each, will consist of young individuals who are recruited during pregnancy into the family planning clinic for postpartal advice. These will be individuals who had never previously taken contraceptive steroids. Group I will consist of 50 women who, by their choice, select contraceptive steroids as the means of family planning. Many in this group will have the added stress of having a baby during their teenage years when they are still growing. Group II, a control group, will consist of women matched for age, parity, socioeconomic status, height and weight, and who choose methods of family planning other than steroids. Group III will consist of approximately 100 women attending the family planning clinics who have used contraceptive steroids for over 2 years. Group IV, a control-group, will consist of 100 women who choose methods of family planning other than steroids, but otherwise are comparable to Group III. Groups I and II will be examined at their 6 week postpartal visit to the clinic and again six months later. These women can be used to determine if nutritional effects are observed in addtion to those related

to pyridoxine and folic acid deficiencies which have been reported by Luhby and others. Groups III and IV will be used to determine whether there are consistent differences in the nutritional state of women who have received contraceptive steroids for a period of two years and those who have not. The increase in the size of these two groups (100 women each) is necessary because only one evaluation will be made with no prior baseline data. Five categories of data will be obtained in all women in each of the four groups: (1) clinical, (2) hematologic findings, (3) serum and urinary vitamin levels, (4) serum lipids and proteins, and (5) tryptophan load tests and blood glucose concentrations.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Interaction of Contraceptive Steroids with Metabolic Functions of Vitamin B_6
Contractor: University of Pennsylvania School of Medicine
Money Allocated: $104,637 (FY 1972 Funds)

Objectives: The purpose of the proposed study is to perform a statistically valid analysis of clinical and chemical disturbance of B_6 metabolism in women on oral contraceptive pills and in pregnant women. This should lead to the establishment of necessity of B_6 supplementation in the above two conditions and to the desirable dose level of supplementary pyridoxine. Accompanying clinical conditions, like depression, edema, rheumatoid manifestations, will receive special attention.

Significance to Biomedical Research and Program of the Institute: The proposed project may provide information on mechanism and consequences of altered vitamin B_6 metabolism in contraceptive users. In all published studies on abnormalities of vitamin B_6 status resulting from use of contraceptive steroids and pregnancy, primary or exclusive reliance was placed on the tryptophan load test or the determination of blood transaminases. This was due to lack of precise, reliable and reproducible analytical procedures for estimating pyridoxal phosphate (PLP) directly. In 1970 Chabner and Livingston described a method for the estimation of plasma PLP based on the measurement of $^{14}CO_2$ evolved from the decarboxylation of l-tyrosine-1-^{14}C by tyrosine decarboxylase. The amount of $^{14}CO_2$ produced is proportional to the amount of PLP added to an excess of tyrosine apodecarboxylase. A modification of this procedure to be employed in the proposed project may offer new insight in the relationships between contraceptive use, primary manifestations of altered B_6 status and its secondary consequences, such as an abnormal tryptophan metabolism.

The dosage of pyridoxine supplementation is a pharmacological one and greatly above what is known as the requirement for this vitamin. Such a high dosage may be necessary to correct all abnormalities in tryptophan metabolism in all contraceptive users. This provides for simplicity of experimental design but limits reliable estimation and interpretation of altered dietary requirements of this nutrient. The experimental design underlying the project is considered outstanding both in regard to its epidemiological-clinical components and the nutritional-biochemical methodology.

Proposed Course: Three groups (35 in each group) of properly selected women will be studied to assess the metabolic interaction of contraceptive steroids and vitamin B_6. Group A has received previously oral contraceptives. Groups B and C have not used oral contraceptives before. After enrollment all women in all groups will undergo clinical examination. Simultaneously chemical studies will be carried out in the blood (plasma) for pyridoxal phosphate (PLP), in the erythrocytes for GPT, GOT, and in tryptophan-methionine load tests for xanthurenic acid and kynurenic acid in the urine and for homocysteine, cystathionine, and taurine in the blood. In patients showing signs

of depression, serotonin will be determined in the blood. Since the patients in Group A have been for at least six months before entrance in the study on contraceptive steroids, it is to be expected to find in them pathological disturbances of the B_6 metabolism, whereas Groups B and C never before receiving oral contraceptives should behave as normal controls. After completion of the initial clinical and chemical studies, Group A will continue and the second group will begin to take a combination oral contraceptive in the usual 21-7 day cycle. The third group will receive minidose progestogen alone. Groups A and B will receive 100 mg. pyridoxine HCl daily during the 7-day interval between active contraceptive medication. Repeated clinical and chemical studies will be made at 1, 3 and 6 months after entrance into the study. The probands for the three groups of contraceptive users are available from the Hahnemann Medical College and Hospital Family Planning Clinic. The two groups of pregnant subjects will be supplied through the private practice of the co-principal investigator.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Evaluation of Effects of Oral Contraceptives on Vitamin B_6 Nutrition
Contractor: University of Wisconsin Medical School
Money Allocated: $99,657 (FY 1972 Funds)

Objectives: The objectives of this study are to determine if the use of oral contraceptives enhances the rate of induction of vitamin B-6 deficiency in women on a defined intake of vitamin B-6 and folic acid, and to determine accurately the amount of vitamin B-6 required to correct the metabolic alterations observed.

Significance to Biomedical Research and Program of the Institute: Altered tryptophan and vitamin B-6 metabolism appears certain to occur in a number of women ingesting oral contraceptives whether these alterations have significant consequences to impaired health remains unclear. The studies outlined in this proposal represent an attempt to provide information concerning this problem. Eventually much larger population groups would need to be studied. Results of this project may be expected to delineate those aspects that would be of importance in such a population study. It is therefore highly relevant to the program. Since prior studies of vitamin B-6 metabolism in pregnancy and conditions of illness have not considered or controlled intake of the vitamin, clarification can be expected through use of healthy subjects under conditions of depletion and repletion.

Proposed Course: It is proposed to study vitamin B-6 metabolism in 24 healthy young women. Six would serve as control subjects who have not used oral contraceptives. The remaining 18 subjects would represent women who have regularly used oral contraceptives for at least 6 months. All subjects will be placed on a defined diet providing daily 75 gms of protein and 0.16 mg of vitamin B-6. The nutritional and metabolic functions related to vitamin B-6 and folic acid will be assessed initially and at intervals while consuming the deficient diet. The rate of induction of metabolic deficiency will be compared between the controls and those receiving the steroids. Following a deficiency period of 28 days, the subjects will be repleted for 28 days with known amounts of vitamin B-6. The control subjects will receive 0.8 mg of additional pyridoxine daily, while the oral contraceptive users will be divided into 3 groups and receive either 0.8, 1.5 or 15 mg of additional pyridoxine daily. Upon completion of this period, all subjects will be supplemented with 20 mg of additional pyridoxine for 5 days. The metabolic studies will be conducted at comparable phases of each subject's menstrual cycle in order to avoid possible cycle-related metabolic variations. These studies will include measurement of tryptophan metabolites following a tryptophan load, measurement of cystathionine and other amino acids following a methionine load, measurement of urinary creatinine, 4-pyridoxic acid 5-hydroxy-indoleacetic acid, 3-methoxy-4-hydroxymandelic acid, measurement of plasma kynurenine, folic acid and pyridoxalphosphate and measurement of erythrocyte transaminases. Mental status

will be evaluated through the use of a self-administered test. Electroencephalographic examinations will be administered on each subject before depletion, during depletion, and after resupplementation with pyridoxine. Kynurenine load tests will be used to determine whether or not any altered tryptophan metabolism is due to a vitamin B-6 deficiency or to elevated tryptophan pyrolase activity.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title : Interaction of Contraceptive Steroids with Essential Dietary Nutrients
Contractor: Wayne State University School of Medicine
Money Allocated: $68,090 (FY 1972 Funds)

Objectives: The purpose of the epidemiological clinical study is to characterize nutritional profiles of selected and well-defined sub-populations of the Detroit area belonging to different socio-economic levels and users of oral contraceptives. Specifically, status of trace elements and vitamins will be investigated in this study. A correlation between the abnormal nutritional parameters and use of oral contraceptive pills will be sought by including proper controls of similar socio-economic backgrounds who are not using any oral contraceptives. Nutritional status of all subjects will be determined by proper physical examination and detailed dietary history. This will provide a basis for proper evaluation of changes due to use of oral contraceptives per se and interaction between the pills and poor nutrition on various abnormal parameters can be studied.

Significance to Biomedical Research and Program of the Institute: The scientific merit of this project is in its broad coverage of most of the important nutritional factors possibly interacting with oral contraceptive drugs. This study will produce useful information on the effects of contraceptives under the conditions of everyday life, against the background of a good dietary history in two socio-economic classes. The nutrients to be determined are well chosen. The dietary histories will be of value not only in the context of the contraceptive actions but also to nutrition in general. From a nutritional point of view, it is essential to assess any pharmacological effect against the background of dietary history and the conditions of every day life. Even the most sophisticated studies on a metabolic ward have to be extrapolated to conditions outside of the ward. The proposed project will produce a large amount of useful information. The in depth studies, as well as the animal experiments, are directed toward important questions. Consequently, the whole project is highly relevant to the objectives of the program.

Proposed Course: Eight hundred subjects are to be included in the epidemiological study. The subjects will be divided according to their socio-economic background into two major groups and each group will then be further subdivided into four subgroups, i) those who are not taking any contraceptive pills, ii) those taking 1 mg progestogen + 50 µg mestranol, iii) those taking 0.5 mg progestogen + 50 µg ethinyl estradiol and, iv) those women who resume oral contraceptive pills after a pregnancy and during lactation. Women who become pregnant after discontinuation of contraceptive pills will also be included in this study as a special group.

Physical examination for signs of nutritional deficiencies, detailed dietary history, hematological examination and biochemical tests include plasma--

vitamin A, ascorbic acid, folic acid, vitamin B_{12}, zinc,copper, iron magnesium calcium and cadmium, and red blood cells--folate, zinc, copper and magnesium will be carried out. These data will be correlated with whether or not the subject are users of oral contraceptives, their nutritional status, socio-economic background and evidences for interaction of contraceptive steroids with essential dietary nutrients will be looked for.

Approximately 450 subjects will be investigated in the first year, thus two years will be required to complete this phase of the studies. In the third year 15 subjects from each of the groups will be investigated in depth with respect to the changes in trace elements folic acid and vitamin B_6 metabolism. The physiological significance of a decrease in serum zinc level will be investigated further in order to correlate the total body store of zinc with the serum level. FIGLU excretion following histidine loading, oral absorption tests by using purified polyglutamate folic acid and tryptophane metabolites in urine after proper loading doses of tryptophane will be determined. These studies will provide additional data concerning the nature of abnormalities encountered in the metabolism of micronutrients due to the use of contraceptives.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: The Effects of Contraceptive Steroids on Trace Elements
Contractor: University of California at Berkeley
Money Allocated: $70,362 (FY 1972 funds)

Objectives: Definitive information pertaining to the effect of oral contraceptive steroids on trace elements is missing. While many observations have been made pertaining to the influence of OCA on iron, copper and to a lesser extent, zinc metabolism for the most part these observations are directed towards determination of blood levels without regard to trace mineral balances. The objectives of the project are to investigate the effects of contraceptive drugs on trace elements, balance of iron, copper and zinc in women kept on controlled diets in a metabolic ward.

Significance to Biomedical Research and Program of the Institute: The proposed study of iron, copper, and zinc is justified and desirable. These three trace elements are known to be essential. For two of them (iron and zinc) we suspect or know that nutritional problems of suboptimal intake exist in some population groups, and all three are known to be in some way influenced by contraceptive drugs. The proposed balance studies will not only better define a number of metabolic changes, but they can be expected to furnish much needed information on the minimum daily requirement for these trace elements under normal conditions and with contraceptive medication. Only through carefully controlled metabolic studies within a metabolic ward can one accurately define the influence of contraceptive steroids upon mineral nutrition. The investigators possess one of the few unique capabilities in this country to conduct such detailed studies and the staff with the knowledge to provide adequate supervision for such a demanding project.

Proposed Course: It is proposed to conduct a rigidly controlled metabolic study of 6 young normal adult female graduate students who will be maintained under rigidly controlled conditions in a metabolic facility for 17 weeks. They will be fed a constant formula type diet containing all known essential nutrients. Observations to be made will be analysis for all losses including urinary, fecal, skin and menstrual losses; intakes will be determined by analyses of the diet. The subjects will receive in a randomized fashion two months of no steroidal agent, one month of a sequential type oral contraceptive and one month of a combined estrogen-progesterone agent. In addition to studies of trace elements, they will also further evaluate the effects of contraceptive steroids on calcium, magnesium, and nitrogen balances. These balances will be particularly observed in regard to cyclic variations. Blood samples will be obtained and analyzed three times per menstrual cycle. Basal metabolic rates and requirements to perform a fixed amount of work will be determined throughout the cycle. Body weight variations will be observed. The trace mineral balance studies are primarily concerned with iron, copper, and zinc, employing conventional methods of analysis (absorption spectrometry for zinc and copper and calorimetric analysis for iron).

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Workgroups on Contraceptive Drugs
Contractor: Drug Research Board, National Academy of Sciences, National Research Council
Money Allocated: $25,000

Objectives: To conduct a series of workshops on various subjects concerning the safety of steroid contraceptives and related methods of chemical contraception. Specifically, six workshops will be conducted over a two-day session immediately following the Fifth International Congress on Pharmacology in San Francisco during July 1972. Each workgroup will be open to a restricted audience and will be expected to prepare a report on the status of research in its respective field. The titles of the workgroups are: Cancer, Epidemiolo Pharmacokinetics and Metabolism, Prostaglandin, Lipids and Renin-angiotensin, Problems in Regulation. It is expected that the report will be submitted to the Drug Research Board and subsequently to the Center for Population Research for guidance in program planning.

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Center for Population Research
Fertility Regulating Methods Evaluation Branch

Contract Title: Symposium on Chemical Contraception
Contractor: Federation of American Societies for Experimental Biology and Medicine
Money Allocated: $12,500

Objectives: To conduct a one-day symposium on chemical contraception as part of the general program of the Fifth International Congress on Pharmacology to be held in San Francisco during July 1972. A series of presentations representing a review of the status of this field will be presented in a general session as part of the Congress. The various subjects to be covered include: points of vulnerability, steroidal agents, non-steroidal agents, intrauterine devices, male agents, future developments, pharmacokinetics of steroid contraceptives, problems of experimental design, metabolic effects, cancer, vascular system effects and epidemiology. It is anticipated that this comprehensive review of the field will be of considerable interest and stimulating to the pharmacologists attending this meeting.

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Fertility Regulating Methods Evaluation Branch

Contract and collaborative projects listed below are reported in detail in the Biometry Branch reports on pages indicated.

A Collaborative Study of Oral Contraception and Cerebrovascular Disease..... BB6

A Comparison of the Medical Effects of Induced Abortion by Two Methods, Curettage and Suction BB7

Oral Contraceptives and Tumors of the Breast ..... BB8 - 9

A Retrospective Study of the Risks for Cancers of the Breast, Body of the Uterus, Ovary, and Cervix Among Users of Oral Contraceptives..... BB10

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Office of the Associate Director for Extramural Programs

The four Branches within the Extramural Programs of the Institute (Adult Development and Aging, Growth and Development, Mental Retardation, and Perinatal Biology and Infant Mortality) have endeavored to maintain high quality research and training programs during fiscal year 1972 in spite of the fact that increasing numbers of approved applications cannot be funded. This actually makes the work of the scientist administrators more difficult than during periods of less financial constraint. The reasons are many but include the fact that we are still anxious to engage in "active programming" of good applications, but at the same time must be careful not to give any applicant the impression that he is assured of being funded just because we are interested in his research and in having him make an application for support. The work load is also greatly increased because when requested we try to provide disappointed applicants (whether disapproved or approved but not funded) with full and accurate critiques of their applications. In addition to these two very demanding tasks, our scientist administrators continue their active involvement in planning and running conferences, many of which result in important scientific publications for which the scientist administrator is responsible. The high social relevance of the type of research and training supported by the NICHD makes it all the more important that we continue to support these conferences and publications. It may also account for the fact that the number of research grant applications continues to increase in spite of the fact that research grant funds increase far more slowly.

Previous annual reports have mentioned the excellent working relationship which exists between scientist administrators and grants and contracts managers within the Institute. I wish now to emphasize the fact that in difficult times such as these the value of dividing the work load as we do becomes ever more apparent. With the close cooperation which exists, the system is both highly efficient in terms of productivity and eminently successful in conserving the time of both the scientist administrators and the grants and contracts managers. The scientists are able to concentrate on the substance of their science because they know that they can depend upon the grants and contracts managers for expertise in the legal, fiscal, regulatory and procedural aspects of the support methodology. Important decisions regarding grants and contracts are always made jointly. Extremely close cooperation must and does exist between the two staffs at all levels, including the two Associate Directors involved (Extramural Programs and Program Services). The advantages of the system are so great that we find it hard to understand why after more than eight and one-half years of successful operation, no other Institute at the NIH has adopted it.

The NICHD Research and Training Committees established some years ago continue to function smoothly. Their major problems have centered around the review of program projects. The complexity and large size of most program projects inevitably makes their review difficult. As the shortage of funds has become more serious, the Committees are increasingly adopting

the practice of cutting out mediocre sub-projects before approving program projects. They are also looking more and more critically for the "glue" which is supposed to hold a program project together, that is, the overall unity of research goal and the clear interdependence of the sub-projects. The Committees are fully aware of the fact that a program project must be more than the sum of its various parts.

During fiscal year 1970, the National Advisory Child Health and Human Development Council became concerned that the growing portfolio of program projects might threaten the support of regular research grants. After full discussion, they instructed the Associate Director for Extramural Programs and the five Chairmen of the Research and Training Committees to present a plan at the June 1970 Council which would assure the safeguarding of quality control of program projects. The plan presented and approved unanimously by the Council involves a full scientific review every three years of every program project and center grant, beginning with applications which met the October 1, 1970, deadline. Continuing Council concern led to another discussion during the March 1972 Council meeting at which the Associate Director for Extramural Programs expressed confidence in the five Research and Training Committees, especially with regard to their determination to uphold high standards of excellence. Council was assured that staff and committee members will continue to scrutinize each program project with great care in an effort to make certain that only high quality work is being supported. In addition, the percentage of NICHD research grant money devoted to the support of program projects will be announced at each subsequent Council meeting.

Beginning in fiscal year 1972, the initial review of research career development award applications was transferred from the Research and Training Committees to DRG Study Sections. The new system allows an RCDA applicant to be reviewed by the same Study Section which is responsible for his research grant application. Also, the former dual review of fellowship applications was discontinued by the NIH and the full responsibility placed on the Research and Training Committees. It was becoming clear that dual review of fellowship applications was an unnecessary waste of both academic scientific manpower and NIH staff time. Committee members and staff adapted well to the changed circumstances, and it would appear that the new arrangements are working out very satisfactorily.

An important liaison exists between the Population and Reproduction Grants Branch within the Center for Population Research, NICHD, and the Extramural Programs. Technically, the Branch Chief reports to the Director of the Center; in terms of actual operation, all important decisions are also discussed with the Associate Director for Extramural Programs. In addition, the Branch Chief regularly attends all meetings of Branch Chiefs called by the Associate Director for Extramural Programs. It is a pleasure to report that the relationship is an excellent one, resulting in uniform extramural policies.

Extramural scientific staff are more and more heavily involved in the development and supervision of research contracts. The requirement that requests for proposals must be announced has been added to the work load,

but in all fairness it seems to us that the quality of proposals has increased. The required monitoring of contracts at all stages, however, does represent a very considerable investment in time and effort. That staff members are willing to devote this time and energy is testimony to their conviction that the missions of the Institute are definitely enhanced by the research which can be accomplished through the contract mechanism.

Two valued staff members left the Extramural Programs during fiscal year 1972. Dr. Gabe Maletta left the Adult Development and Aging Program to enter medical school, and Dr. Mark Fitzpatrick left the Perinatal Biology and Infant Mortality Program to return to private practice. Dr. G. Eric Knox ably took over Dr. Fitzpatrick's duties from January 1 to June 30, 1972, at which time he leaves to continue residency training. A successor has not been located. We were extremely fortunate to find a replacement for Dr. Maletta in Dr. Lester Smith who joined the Adult Development and Aging Program during July 1971. In the Growth and Development Program, Dr. George A. Morgan assumed the duties of Executive Secretary to the Research and Training Committee, a position which for some months had been filled by Dr. James F. Kavanagh in addition to his already very heavy work load. This example of the willingness of one extramural scientist administrator to assume demanding duties in addition to his normal duties is typical of the extramural operation in general.

The significant activities and accomplishments of the extramural branches are summarized in the reports which follow.

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Growth and Development Branch

The Growth and Development Branch supported approximately 31% of the research grants in the NICHD during FY 1972. This proportion has remained constant for the past several years.

During this final year the Branch has sponsored three conferences and workshops. Two of these have been devoted to the origins and development of motivation in early childhood; the primary thrust was the creation of new theories and research strategies for this significant research problem. The other conference was, in fact, the sixth in a series on immunology which have been sponsored by NICHD. The June 1972 conference was entitled "The Biological Role of the Immunoglobulin E System." Although immunoglobulin E is a very minor component of serum proteins, its biological function is highly important since it is the major human immunoglobulin capable of mediating reaginic hypersensitivity reactions (such as asthma and hay fever) in man. The proceedings of this conference will be published.

A text entitled Language by Ear and by Eye, co-edited by a member of the Growth and Development Branch staff, was published by the MIT Press at the close of this reporting period. Similar in format to a preceding college textbook in psycholinguistics, entitled The Genesis of Language, the second publication in the "Communicating by Language" series sponsored by the Branch, this book includes papers from a variety of disciplines which examine and contrast the ease with which children acquire speech and the difficulty they have with reading. By comparing the processes which underlie these two forms of linguistic communication and by studying the relation between them, the text should stimulate research into the difficult question of why so many children who learn to speak and listen well have so much difficulty in learning to read and write.

A new publication, Sleep and the Maturing Nervous System, was released by Academic Press in June 1972. This publication is based on the proceedings of a conference which was concerned with the ontogeny of sleep-wakefulness behavior and the basic neural mechanisms which may underlie these patterns of behavior. Current status of knowledge of morphological biochemical and electrophysiological maturation of the brain was explored in this context.

Mechanisms of Biological Growth

The goals of this research area continue to be the elucidation of the nature of the growth process and the exploration of factors and processes which control and promote growth. In scope it extends from basic studies in developmental biology related to growth at the cellular and molecular levels, to physiological and metabolic studies of growth in higher organisms including humans.

The basic studies are fundamental to progress in understanding the growth process, and constitute an important segment of this area. There are ongoing projects exploring the synthesis of proteins and its regulation by genetic,

hormonal and other factors; the development of enzyme systems, their induction, regulation and characterization in different phases of development; the processes regulating cell differentiation, and the structure and function of component parts of the cell during development. One of the new projects initiated during the year will utilize a unique experimental system to study the genetic control of developmental pathways. The investigator has discovered and characterized the genes which control the development of specific segments of the experimental organism. His rationale is to use mutants and certain types of chromosomal rearrangements as genetic tools to dissect the individual component steps of specific developmental pathways. His findings will have broad significance for understanding the genetic regulation or development in higher organisms.

Physiological and metabolic studies supported by this area are chiefly concerned with elucidation of normal growth processes but, as with the basic studies, can contribute to an understanding of impaired growth and development. The major thrust has been in the field of endocrinology: the role of the endocrine system in growth, the mechanisms whereby specific hormones exert their effect, endocrine function as a factor of development, critical periods in development, when hormones exert an effect upon growth, neuroendocrine control mechanisms in growth, and endocrine function in growth-retarded states. A research area identified for greater emphasis has been that of postnatal sexual differentiation, particularly the events which lead to the onset of puberty and regulate its course. The biological mechanisms which underlie this period of rapid growth and profound physiological and psychological change are still to be clarified. Plans have been completed for a conference on The Control of the Onset of Puberty to be sponsored by the Growth and Development Branch in the coming fiscal year. A new project initiated during the year will explore mechanisms of puberty in a variety of experimental animals. It is proposed to study not only the currently favored hypothesis that a changing feedback sensitivity of the hypothalamic-pituitary system to gonadal steriods triggers the onset of puberty but also the possibility that a change in gonadal sensitivity occurs despite previous evidence to the contrary. The program also supports a number of ongoing projects in which pubertal changes are being documented in humans, and mechanisms sought. At a more basic level, the effects of hormonal changes at puberty upon induction and activity of enzyme systems are being explored.

Developmental Immunology

The research supported in this area seeks to clarify developmental events throughout the life span which lead to effective host resistance and to specific immune responses. Both animals and humans are utilized as experimental models permitting insight into basic immune mechanisms. The phylogenetic approach uses mammals and lower animals with less complex immunologic systems in order to identify developmental stages and simpler mechanisms. The ontogenetic approach in humans is also used so as to unravel the regulatory factors which control time-linked changes as they occur with increasing age and maturation from the fetal period onward. The objective is to understand the ability of the human host to combat infection and disease. Although there is no categorical disease orientation, efforts are focused on studies of basic mechanisms involved in humoral and cellular immunity as well

as an understanding of the local secretory immunologic system and the various non-specific protective mechanisms.

An important study of cellular immunity currently is being funded by NICHD. This investigator seeks increased understanding of cellular functions and intercellular relationships with the lymphoreticular system, both as developmental phenomena and as they moderate host responses to internal and external sources of antigenic challenge. Another research team is studying the factors involved in the transfer and mediation of delayed hypersensitivity in man and animals using both in vitro and in vivo methods. In other studies, cord blood lymphocytes are being obtained from neonates at various stages of development and the capacity of such cells to be sensitized by transfer factor and RNA from sensitive adults and to produce migration inhibitory factor in vitro are being investigated. Still another research group is exploring the mechanism of activation of resting lymphocytes, including the factors stimulating or inhibiting the activation and the nutritional requirements necessary to develop a defined medium for metabolic studies. Long-term lymphoid culture lines will be established from normals and patients with genetic or immunologic defects to provide adequate material for the basic study of these defects in vitro. The broad objective of this work lies in the utilization of cultured cells for the study of various aspects of human genetics and immunology.

Future efforts will be directed toward a holistic approach to the development of immunologic competence. All the various defenses employed by the human host will be investigated so that the totality of the mechanisms involved can be better understood, and a picture of immunologic balance or imbalance in health and disease states will emerge. Emphasis also will be placed on expansion of this area to investigate environmental factors influencing the overall ability of the immunologic system to cope with foreign agents and drugs. Conditions such as malnutrition will be studied for the relationships between nutrition and infection.

Developmental Pharmacology

This area of research has gained increased attention in the last ten years. Most early biochemical studies related to developmental periods emphasized how the body handled endogenous or naturally occurring substrates during various stages of immaturity. Now the importance of maturational deficiencies in managing the disposition of foreign or exogenous molecules is being given consideration. Dosage recommendations in use of drugs have recognized the small size of the developing infant and child; but the capabilities of the developing infant to deal effectively with pharmacologic agents has not received adequate attention. The resulting problems may not only be adverse effects from inappropriately large drug dosage, but in some cases may result in inadequate amounts of drugs being administered.

It is therefore important that more knowledge be available on the pharmacokinetics of foreign substances and drugs which are most commonly a problem or in frequent use in treating infants and children.

One approach currently being considered is a drug surveillance project in hospitalized pediatric populations. By this means, the incidence of adverse drug reactions in children can be determined. Children are given different drugs than adults, and they have different mechanisms for handling these drugs because of metabolic differences, differing immunologic capacity, and other factors. This information can be extremely important in assessing drug effectiveness and hazards in relation to the disease entity under treatment.

One new research project represents a coordinated study with a multidisciplinary approach upon the problem of drug dosage in infants and children. Pharmacokinetic data for various frequently prescribed drugs will be obtained in four common disease states seen in the pediatric age group, including such parameters as absorption, distribution, metabolism, and excretion. The data obtained should not only enhance our knowledge of the pharmacokinetics of drugs in disease states which are being studied, but also in the developing infant and child.

Nutrition

The nutrition research supported by the Growth and Development Branch retains a multidisciplinary approach. Emphasis continues to be placed on the role of nutrition in physiological and intellectual development, and the important interactions among nutrition, behavior and environment. Studies of the metabolic, cellular and behavioral factors associated with obesity are another area of program interest.

The Branch has implemented, through grants and contracts, a coordinated series of animal studies investigating the cellular and functional consequences for the central nervous system of prenatal and postnatal malnutrition. Rat studies are seeking confirmation of preliminary data indicating that severe pre- and early postnatal malnutrition may influence subsequent nutrient utilization and the possibility that the consequences of malnutrition may be additive over generations. The subhuman primate is utilized as a model for more direct correlation of malnutrition effects with those in man. One study is measuring the effects of closely quantified protein and caloric restriction of the rhesus monkey during pregnancy on the histological, biochemical and endocrine characteristics, cellularity and cell function of several vital tissues, including brain, of the infant. Another investigator is pursuing a multidisciplinary study of the physiological and behavioral effects of maternal prenatal protein restriction on the infant rhesus. Another researcher is seeking to differentiate the effects of early social deprivation from protein restriction early in life on later social behavior of the rhesus monkey.

In human studies, special attention is being given to appropriate methods for assessing impaired intellectual and behavioral development and for evaluating the sociocultural environment of malnutrition--which in turn may influence mental development. Although emphasis has been placed on elucidation of the developmental consequences of severe maternal and child nutritional deprivation, possible adverse effects of the lesser degrees of malnutrition more commonly found in the United States are also being considered.

Interesting results have been reported by a grantee conducting a retrospective study of the sequelae of the famine which occurred in the Netherlands during World War II. Unusually complete vital statistics and data from armed forces induction records permitted a measure of the influence of maternal nutritional deprivation (starvation) on fetal and postnatal physical and psychological development, and neurological impairment. It was found that maternal exposure to famine resulted in decreased fertility, a small reduction in days of gestation, and a reduction of 200-400 grams in infant birth weight. Boys whose mothers were exposed to famine conditions during gestation did not differ in intellectual and neurological performance from controls at the time of induction into military service.

Definition of growth patterns, cellularity and metabolic processes of individual fat cells and adipose tissues in children and adults is the main thrust of several obesity studies funded by the Branch. Others are directed toward identification of the behavioral, genetic, endocrine and nutritional factors associated with overeating and weight reduction. The relationships of obesity with degenerative diseases in animals is being studied, as is the effects on body composition of weight reduction through diet or exercise.

One grantee has recently reported that in the rat, maternal caloric deprivation during early lactation produced transient reduction in fat cell numbers and size in the offspring whereas maternal restriction of both protein and calories resulted in permanent reductions in depot fat cell number and size in the offspring. These results may have important implications in human obesity in light of a prior finding by the same grantee that obesity in childhood is a function of fat cell number, size, and age at which rapid increase in fat cell number/size is initiated.

Several investigators supported by the Branch are conducting diverse studies with the aim of more clearly defining the role of nutritional factors in cell growth, kidney development and function, body composition, metabolism of dietary amino acids in early life, and the physiology of normal and abnormal growth.

Physical Growth

Physical growth research supported by the Growth and Development Branch encompasses developmental anatomy, cranio-facial growth, skeletal growth and studies in population genetics relating to anatomical development.

Projects supported in the area of developmental anatomy include descriptions of postnatal development of human cerebral cortex, lung growth, ultrastructural morphology of bone, as well as the growth and hypertrophy of various tissues or organs in health or disease. Cranio-facial growth studies are concerned primarily with identifying the genetic, metabolic and endocrine mechanisms involved and examining interrelationships of the different variables of facial growth and dentition. Several investigators look toward the possibility of eventual prediction of individual cranio-facial growth rates and patterns.

Skeletal growth studies include one project developing normative data on the bone weight, density and ash content of normal children. Another project

involves a longitudinal study of skeletal growth of low birth weight and normal birth control children. Still other investigators, utilizing anthropometric data and radiographs from completed and ongoing longitudinal child growth and development studies, are seeking improved methods for evaluation of skeletal development, assessment of skeletal maturity, and the prediction of mature stature.

Developmental Behavioral Biology

This area within the Growth and Development Branch is concerned with the biological substrate and mechanisms that mediate behavioral processes. Basic studies on the effects of early experience upon the developing brain and behavior are continuing. For example, one investigator has studied the sequential development of the neuroendocrine system with regard to maturation of the stress response. It appears that the period immediately following birth is characterized by high activity of the pituitary-adrenal system which is assumed to be a carry over of some prenatal maternal state. In studies on the psychobiology of lactation, this investigator has reported that the lactating female is less aggressive, more emotionally stable in an open field test, and has a lower plasma corticosteroid response to shock-induced fighting, the open field test, and to ether stress than the non-lactating female. The lactating female appears to be particularly responsive to the suckling stimulus while her neuroendocrinological responsiveness to other stimuli are hampered. These findings, if replicated on primates, would have substantial relevance to issues of lactation and breast feeding in human populations.

Another investigator has conducted follow-up studies on the effects of artificial movement stimulation upon isolation reared animals. Laboratory rearing with a moving artificial mother definitely prevented the development of the stereotyped body-rocking characteristic of the mother-deprived rhesus monkey. In spite of this effect, monkeys raised on moving or stationary surrogates were more similar to each other in most respects than either was to a wild-born monkey. Thus, as would be expected, movement stimulation in isolation from other social variables did not eliminate all elements of the social deprivation syndrome. On the other hand, on most tests the animals raised with moving surrogates resembled wild-born monkeys more closely than did animals raised with stationary devices. These studies have important implications for the nature of the sensory environment associated with child-rearing practices.

Learning and Cognitive Development

Human learning and cognition (thinking) play a vital role in all aspects of individual human growth and development, being fundamental to individual interactions with other humans and with the total environment. Because of its importance, the learning process is of major interest to the Growth and Development Program of NICHD. In its present grant holdings, the Branch supports a broadly based, multidisciplinary effort directed towards understanding learning processes. The current focus includes an analysis of the role of the environment at different developmental stages upon the intellectual development of children. A major portion of the current grant holdings emphasizes early

cognitive learning; that is, how information gained from concrete experience is processed, abstracted, generalized, and developed into strategies for problem solving. These studies are investigating the assumption that certain learning experiences are more effective or optimal at certain (critical) stages of development than at other stages of development. Additional research programs are being established to determine what specific learning experiences for any given developmental period will be optimal to maximize intellectual growth and human potential.

In the immediate past special attention has been given to early preschool intellectual achievements and the relevance of such early learning experiences to the development of educational programs that complement and enhance the development of the individual. More recently, attention has been directed toward a period of adolescence where biological and social changes may be accompanied by learning and cognitive changes. Recent studies in the field of learning show the trend toward an increase in an analysis of learning processes and, in particular, the appearance of a new group of researchers who claim "information processing" as their major interest. These scientists are interested in a variety of mechanisms of gathering, interpreting, storing, and retrieving of information. Much of their work comes from computer scientists, and their theories are frequently tested through computer programs, which they hope will simulate the behavior of human beings who are processing, learning, and remembering information. While still in its infancy, work in the simulation of human behavior has made precise some rather fuzzy thinking about how people go about solving problems. To make a successful computer program, all of the fine details must be spelled out and all of the assumptions must be made explicit. For example, programs that solve letter-sequence tasks like those found in intelligence tests, and programs that prove theorems, indicate that the approach is promising and powerful. While the science of information processing already suggests that our task in trying to understand learning and cognition may be very difficult indeed, it is clear that we now have, through our research programs, powerful tests of the adequacy of most theories of learning and cognitive development.

In FY 1971, the NICHD supported 92 research and training grants, fellowships, and contracts in learning and cognitive development. Sixty-four, or about 70% of these grants, were the responsibility of the Growth and Development Branch. While more applications for research support have been reviewed during this year as compared with preceding years, a drop of about 10 grants in our research support portfolio represents the gradual decline in the number of new grants which can be supported by available NICHD funds.

Human Communication

This research area focuses on the fundamental research problems associated with the human communicative process and the role of this process in the growth and development of humans viewed both as individuals and as members of a society. This area examines the biological, psychological and social processes whereby infants and children receive culturally symbolic and non-symbolic stimulation, store and/or associate this information with past experience, and appropriately alter and transmit selected portions of this information to others. At present, special attention is being given to speech

and language development as specific aspects of the communicative process, and to the problems associated with the complex process of visual symbolic reception, perception and association, which may interfere with the normal development of reading skill. During this supporting period, as in the past, this area has encouraged a multidisciplinary approach to many of these problems, and, when appropriate, an interdisciplinary effort on a single researchable topic. As an illustration, on one program project a single team of investigators interested in the speech code, language acquisition and reading include the following backgrounds: physics, psychology, linguistics, psycholinguistics, anatomy, physiology, speech science, communicology, neurology, plus both mechanical and electrical engineering.

Currently there is a strong and growing research interest (in part the result of program stimulation) in the acquisition of language by children, and in how these children develop the capabilities that will be used as adults in communicating via spoken language and in reading written language. Much of this research has been dependent upon specialized tape recordings and technical assistance which is not readily available to researchers interested in language development, and which could cost the user much time and money to acquire. The Branch, therefore, established a contract (described later in this annual report) to make available such specialized services--that is, specialized recorded tapes for dichotic listening studies and research assistance--to competent researchers who were studying the spoken language and its development in children.

As an extension of the Branch's basic biological and behavioral research activity concerning the processes of communication, the Branch has supported a conference series reported in previous annual reports, entitled "Communicating by Language." Through these meetings, and the resultant publications, the Branch has attempted to determine the existing and potential directions of study as well as to identify the areas of needed research. The fourth conference in this series which was held in May, 1971, examined the relationship between speech and learning to read.

Personality and Social Development

Personality and social development of children are concerned with the ways children develop from immature, dependent organisms to fully participating members of society. The Growth and Development portfolio in this area supports 24 research grants plus three contracts and contains some of the most distinguished names in child development research. During the past year, investigators have made various new discoveries. To list but a few, one investigator has found that children begin to make judgments as early as eight months of age. Another investigator has shown that an authoritative (in contrast to authoritarian) style of parental authority is best suited to inculcate certain traits of responsibility and independence in children. Still other investigators have shown that the effects of racially integrated classrooms differ dramatically depending on the attitude of the teacher and the social patterns of each classroom.

During the past year, a complicated, interdisciplinary study has been initiated to assess the etiology and the reversibility of the physical and psychological sequellae of children suffering from maternal deprivation. In another development, a PL 480 project may soon be funded in Yugloslavia to study the relationship between social structure and how families and individuals adapt to modern conditions and needs. This PL 480 research complements an NICHD contract which is currently studying family modernization across five countries.

There were three conferences in the personality and social development area in FY 1972. The NICHD, together with NIMH and the Division of Research Grants, NIH, sponsored a conference on achievement among minority groups. The Growth and Development Branch also supported two workshops on the development of motivation in childhood. The first workshop concerned itself with the development of motivation in early childhood and concluded that motivation is still a viable concept but that it should be treated as an individual-social structural "systems variable" rather than as an individual trait. The second workshop looked at the developmental course of specific motivations.

Training for Research

The Growth and Development Branch supports three types of research training programs: training grants, fellowships, and research career development awards. During FY 1972, a total of about four million dollars will be spent on growth and development training in support of some 350 trainees in the three programs. About 70% of the funds will go to support the pre- and postdoctoral training grants, 5% will be for postdoctoral and special fellowships, and 25% will be for RCDAs.

The overall dollar support for Growth and Development training has remained at approximately the same level since 1967, but the number of trainees and the proportion in each of the three types of program has varied considerably. For example, the number of Growth and Development trainees grew steadily from the establishment of the Institute until it reached about 450 in FY 1968. Since then the total has declined to less than 350 because of a marked drop in fellowships in 1970 and a gradual decrease in the number of stipend holders on training grants, where the total direct costs have been relatively constant but the increasing cost of faculty salaries, supplies, and equipment have cut into the amount going into stipends. In general, the above trends for the Growth and Development Branch parallel those for the Institute as a whole. However, the G&D Branch appears to have suffered more than most in recent years as indicated by the fact that the proportion of NICHD training monies going to G&D has declined from about one third in FY 1967 to only about one quarter this year.

In March 1972, there were 38 active growth and development training grants, split about 60-40 between biomedical and behavioral programs. This is a marked change from March 1966, when 80% of the 39 training grants were for biomedical programs. A related change has taken place in the shift from training predominantly postdoctoral fellows in the early years of the Institute to about two thirds predoctoral trainees during FY 1970.

The 22 active biomedical training grants are almost entirely for post-M.D. research training. Most of them are broad programs offering training in several areas or subspecialties within a pediatrics department; however, a number of the G&D biomedical grants are more focused offering training in areas such as human genetics, developmental immunology, or kidney and electrolyte physiology. One grant attempted to combine training in pediatrics and psychology. The 15 behavioral grants are predominantly for predoctoral training in developmental psychology, but the Branch also supports programs in areas such as developmental psycholinguistics, human learning, and the sociology of socialization.

In FY 1972 only two new training programs were funded. One of these was an interdisciplinary predoctoral program entitled "Cognitive Development in Early Childhood." The other new training program also was interdisciplinary providing pediatricians and other qualified applicants with research training in developmental medicine. Training is provided by members of the pediatrics, medicine, and surgery departments in areas of experimental embryology, developmental biochemistry, and developmental genetics.

As of March 31, 1972, the Branch supported 19 fellowships, less than half the number of two years ago, but a somewhat higher number than last year. In contrast to training grants where the Growth and Development Branch trains very few basic biologists, a majority of G&D fellows have Ph.D.s in some area of biology. The remaining fellows are about equally divided between behavioral scientists and physicians. The 39 active RCDAs cover a range of biomedical and behavioral scientists working in growth and development. The number of G&D RCDAs has remained quite constant since 1966, but the percentage of behavioral scientists has increased somewhat to about one third of the total.

Special Problem Areas and Program Plans

The major problem in developing research programs in the growth and development area continues to be limitations on funds. This has been partially ameliorated during this fiscal year by the budget increase permitting funding of approximately 50% of the approved grant requests. The support of training for research careers has not been as satisfactory, however. Unfortunately, about a dozen former G&D training programs were not refunded and have been terminated during FY 1972. Because there seems to be little immediate prospect of an increase in training funds, perhaps a larger proportion of training grant funds should be put into stipends so that the trend toward a decreasing number of trainees and training programs could be reversed or at least stemmed. One way of accomplishing this shift would be to limit the amount of money for salaries, supplies, equipment, etc., which the Institute would pay per trainee, in much the way the supply allowance is now handled for fellowships.

It has become increasingly apparent to the Branch staff that development during adolescence is inadequately represented in NICHD grant holdings. This gap parallels a stagnation in research on this subject throughout the United States in recent decades. Nevertheless, this is a period when profound

physiological changes take place in the individual, when a major growth spurt occurs, and when critical adjustments in individual and social behavior are required. The staff is developing a major new coordinated research program to clarify this confusing but important area. Grants, contracts, conferences, and preparation of new reference texts will be included in this expanded multidisciplinary effort.

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Growth and Development Branch
Contract and Collaborative Research

Contract Number: N22 02241
Contract Title: Methodology for the Study of Nutrition as Related to Mental Development and Brain Function
Contractor: St. Joseph Hospital, Lancaster, Pennsylvania
David B. Coursin, M.D.
Money Allocated: None in FY 1972

The objective of this contract has been to develop standardized test procedures and instrumentation for the electrophysiological assessment of sensory capacity and function for the visual, auditory and somatosensory modalities. The nature of this testing protocol is being designed to maximize information about cortical brain function as it relates to complex cognitive processes. Specific objectives include fabricating a portable multi-sensory (visual, auditory, somatosensory) testing module that can be utilized for field nutritional studies. This module will have timing circuits and programs that will allow multiple scheduling of sensory stimulation.

The contractors have successfully completed the following tasks:

1. Evaluated all commercially available pieces of apparatus that might serve as component parts of the system. Have selected those most applicable and made appropriate modifications in order to adapt them specifically for our needs.

2. Acquired a prototype of the Barlow Curvereader (manufactured by Shipton in Iowa) and subjected it to rigorous testing that encompassed the parameters of performance required for the system. These tests showed conclusively that the apparatus has definite limitations:

   a. Optimum threshold setting of the system depends upon paper speed and must be adjusted continuously.

   b. The system does not reproduce rapid fluctuations (ER's) in the EEG.

   c. Significant distortion is evident at EEG levels greater than 1.5 centimeters.

Extensive consultation with Barlow and Shipton revealed that they have had similar difficulties and have been unable to improve the quality of reproduction to the level that would be required for contract purposes. This particular method that had been originally thought applicable has been eliminated. A more accurate and completely satisfactory technique has been developed.

3. An electronic control mechanism for the system using cassette recorded guidance has been contructed and, with the recent advent of computer quality cassettes, it has been possible to adapt this procedure for the electrophysiological data recording mode. Tests of its performance have been completely satisfactory with demonstrated reproductive quality and flexibility that easily encompasses the range of requirements for the system. An excellent means for acquiring data directly on cassette tape in the field and converting this to the computer magnetic tape with appropriate time codes has been developed.

4. Overview:

a. Stimulation and recording apparatus are in their final stages of construction.

b. The Ampex FR 1300 tape recorder has been preferred to the new Ampex 2000 series and a comparable Honeywell device.

c. Preparing for trial runs with patients in the laboratory has been initiated to gain practical experience in the use of the system.

Contract Number: N01 12419
Contract Title: Effects of Calorie and Protein Restriction in Pregnancy and Newborn Rhesus Monkeys
Contractor: The Johns Hopkins University, Baltimore, Maryland
Donald B. Cheek, M.D.
Money Allocated: None in FY 1972

The research conducted under this contract represents the NICHD contribution to a collaborative NICHD-NINDS study to measure the possible effects of protein and calorie restriction during pregnancy in rhesus monkeys on selected histological, biochemical and endocrine characteristics of their infants. Four dietary regimes are being imposed: normal protein and calorie; low protein-normal calorie; normal protein-low calorie; and low protein-low calorie. Care of the females and delivery of the infants by cesarian section is being carried out by NINDS staff. The NICHD contractor is conducting a series of laboratory determinations on vital tissues of the infants to measure the degree of anthropometric, histologic, biochemical and endocrine damage, if any, imposed by the experimental maternal diets. Analyses include estimates of cellularity and cell function of the brain, muscle, liver, and various endocrine glands.

A pilot study is now underway. The results will be evaluated by a consultant advisory committee prior to initiation of the main study. This review of pilot study results will be conducted late in the summer of 1972 and it is anticipated that the main study can be undertaken in September 1972, if warranted by the pilot data now being obtained.

Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01 22027
Contract Title: The Reversibility of the Physical and Psychological Sequellae of the Maternal Deprivation Syndrome
Contractor: Wayne State University, Detroit, Michigan
Charles Whitten, M.D.
Money Allocated: $175,371 (FY 1972)

This is a longitudinal multidisciplinary study designed to test the major etiological hypotheses of the maternal deprivation syndrome in infants and to evaluate an intervention program constructed to reverse and eliminate the symptoms. Specifically, it aims: (1) to determine the extent to which the physical and psychological retardation associated with the maternal deprivation syndrome can be reversed, (2) to determine whether the retardation in physical growth sustained by maternally deprived infants is the result of an inadequate intake of food or is secondary to psychological factors operating with the child which adversely affect endocrine control over growth, and (3) to obtain a clearer understanding of the specific cognitive deficits associated with the maternal deprivation syndrome.

Contract Number: N01 12417 (FY 1972)
Contract Title: Ecology of Malnutrition in Vulnerable Groups
Contractor: Massachusetts Institute of Technology, Cambridge, Massachusetts
Nevin S. Scrimshaw, M.D., Ph.D.
Money Allocated: $136,025

The goal of this contract is to determine what factors in the life of low income families lead to the development of malnutrition in preschool children. The methods to be used are essentially multidisciplinary ones built around the epidemiologic approach to identifying contributory factors in families having one or more malnourished young children.

During the first year, the contractor is to concentrate on pilot testing the methods to be used in a proposed one-two year definitive study. Special attention is to be given to the development of appropriate sociological methodologies and to testing these in families living in the inner city area of Cambridge, Massachusetts. Further documentation concerning the existence and nature of malnutrition in children from this community will be developed. The proposed new methods and the results of a pilot study are to be presented at the end of the first contract year.

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Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01 92257
Contract Title: Study of Chemical Composition of the Human Placenta
Contractor: University of Iowa, Iowa City, Iowa
L. J. Filer, Jr., M.D. and Samuel Foman, M.D.
Money Allocated: None in FY 1972

The objective of this project is to do a comparative study of the composition and cellularity of placentas, coupled with newborn birth weight, from full-term uncomplicated pregnancies in well nourished and poorly nourished populations. The following types of placental studies are being pursued:

1. Evaluation for water content, fat, protein, and hydroxyproline

2. Determination of trace element concentrations

3. Calculations of cellular characteristics

    a. Total numbers of cells

    b. Indices of cell size

    c. Indices of cell mass

4. Comparisons of placental/newborn characteristics

This contract involves collaboration of four different institutions. These include The University of Iowa, University of Washington, Tulane University, and the Institute of Nutrition for Central America and Panama. Individual analyses have been carried out on all the specimens received, with some placental specimens still to be collected. This contract is to terminate in September 1972, at which time data analyses will be complete on biochemical evaluations of placentas. This information will then be analyzed in relation to nutritional status of mother and characteristics of the newborn, and a report on this study will be published in final form.

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Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01 22069
Contract Title: Immunologic Responses of Normal and Malnourished Infants to Standard Antigens
Contractor: University of California, Los Angeles, California
Charlotte G. Neumann, M.D., M.P.H. and Richard Stiehm, M.D.
Money Allocated: $68,100 (FY 1972)

The main objective of the project is to study humoral and cellular immune responses to standard antigens in normal and malnourished infants, with special emphasis on identifying changes due to nutritional deficiencies. The UCLA laboratories will develop methods for immunologic and nutritional assessment of malnourished populations in Ghana, Africa, which will be pre-tested on a small sample of North American children. Well nourished controls for the malnourished children will be selected on site in Ghana.

Four groups of subjects will be studied:

1. Hospitalized Ghanian children with severe malnutrition
2. Ambulatory Ghanian children with moderate malnutrition
3. Age-matched Ghanian children without malnutrition
4. Age-matched U.S.A. children without malnutrition

The testing to be employed consists of:

1. A battery of immunologic testing including studies of deficiencies in antibody immunity, cellular immunity, granulocyte function, and opsonic function.

2. Assessment of nutritional status to include clinical nutritional assessment, anthropometric measurements, and biochemical assessment of nutrients.

3. Other studies to include routine hematologic studies, thick blood smears for malaria, stool exam for ova and parasites, and sickle cell preparations.

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Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01 12088
Contract Title: Non-Human Primate Breeding Colony
Contractor: University of California, Davis, California
Andrew G. Hendrickx, Ph.D.
Money Allocated: None in FY 1972

The purpose of this contract is to develop a resource of subhuman primates reared in a standardized environment and of known medical, reproductive and genealogical history, in order to provide research animals of known quality to investigators whose research is supported by the NICHD.

The colony is being developed at the National Center for Primate Biology at the University of California at Davis. Animals of many stages of development including dated pregnancies, embryonic and fetal material, neonates of known gestation, mother-infant pairs, juveniles of known age, as well as certain biopsy material, tissues and fluids, are being made available to selected investigators, all of whom must be directly supported by NICHD. Application for animals is competitive and allocation is determined by an advisory committee which also advises on colony and contract management. Animals are shipped to recipient scientists throughout the country, and in addition a limited number of investigators can be accommodated in visiting scientists facilities on-site. Funds from the sale of animals will be used to offset the costs of future contract years. As the colony is developed, biological and behavioral data profiles are being developed and made a part of each animal's computerized record. Such information can be made available to recipient investigators upon request.

In the second year, further stabilization of the base colony of 250 rhesus monkeys and their offspring will occur. There will be special emphasis on the development of a preventive medicine and health care program for the animals. Some expansion of the breeding program may be undertaken after stabilization is achieved.

As the availability of this resource becomes more widely known, an increasing number of requests for animals have been received from NICHD supported investigators. There is particularly apparent a need for timed pregnancies. Currently 30% of the breeding colony is devoted to the production of closely timed pregnancies. This proportion will be flexible to future requirements.

Growth and Development Branch
Contract and Collaborative Research

Contract Number: N22 81512
Contract Title: A Study of Malnutrition and Mental Development in Bogota, Colombia
Contractor: Harvard University, Boston, Massachusetts
Frederick J. Stare, M.D.
Money Allocated: $75,000 (U.S.-Japan Cooperative Medical Science Program Funds FY 1972)

This project is investigating the effect of protein-calorie malnutrition in early childhood on intellectual and behavioral development of children living in an urban slum in Bogota, Colombia. The design calls for identification of pairs of siblings, both under four years old, one of which is malnourished and the other well nourished. Both siblings will be supplemented with a locally acceptable food for a five-year period; nutritional, anthropometric, and behavioral tests will be done at intervals. Comparisons will be made between siblings at stated ages and between experimental and control populations. In contrast to other studies, this plan mimimizes genetic and social differences between experimental and control subjects.

During this contract period the major scientific direction of the project was modified through the appointment of a new scientific director and the addition of behavioral and data analytic staff. The full pilot study was implemented in June 1971, following the plans reviewed by the outside panel of experts in 1970. The preliminary data from the pilot study have been analyzed and have formed the basis for revisions in the proposed longitudinal study design. The latter has been submitted as a grant application to NIH; a site visit is scheduled in Bogota to review this application. Plans are also underway for analysis of the data from the pilot study simultaneously with the initiation of the proposed longitudinal investigation.

Contract Number: N01 02303
Contract Title: Foster Care: Potential and Self and Public Images
Contractor: Social Psychiatry Research Associates, San Francisco, California
Dorothy Miller, D.S.W.
Money Allocated: $11,200 (FY 1972)

This contract is supporting analyses of previously collected data on the self-image and the public images of foster parents. It attempts to find out the potential which exists in the general population for the recruitment of new foster parents and to validate existing criteria for the selection of foster parents.

Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01 22735
Contract Title: Prediction of Mature Stature in Growing Children
Contractor: Fels Research Institute, Yellow Springs, Ohio
Alexander F. Roche, M.D.
Money Allocated: $104,424 (FY 1972)

This two-year contract has the objective of achieving improved reliability in predicting mature stature of growing children. The prediction tables currently in use were developed from data collected 30-40 years ago and were validated against a very small sample. The contractor, utilizing radiographs and anthropometric data from the three largest longitudinal growth studies conducted in the United States, is developing and validating better parameterization of individual growth in stature from birth to eighteen years.

The contractor conducted this research for a period of two years with grant support. The current contract provides for continuation and completion of the study. Collection, standardization, computerizing and analysis of the data have progressed in accordance with the original timetable. Several factors influencing the replicability of assessments of skeletal maturity with the current tables have been identified. A comparison of prediction errors based on sets of cross-sectional versus serial data is currently underway. Upon completion of the new parameterization of growth in stature, additional analyses leading to further reduction of prediction errors will be undertaken.

Contract Number: N01 80972
Contract Title: Cross-National Research on the Family
Contractor: Case Western Reserve University, Cleveland, Ohio
Marvin B. Sussman, Ph.D.
Money Allocated: $40,000 (FY 1972)

This contract involves the development of theory and methodology in the relationship between: social structure (modernization), family structure, socialization and the outcomes of the socialization process. The aim is to find out how various types of families function at different levels of modernization so that individual family members gain access to educational, health care, occupational and other institutions and thus achieve overall social competence.

Arrangements have been made with collaborators in seven countries who are cooperating with the project staff in the testing of relevant theory. Data gathering is now complete and data analysis is underway.

Growth and Development Branch
Contract and Collaborative Research

Contract Number: NO1 81412
Contract Title: Mathematical Quantification of Developmental Electrophysiology of Brain Function (EEG)
Contractor: Tulane University, New Orleans, Louisiana
Bernard Saltzberg, M.Sc
Money Allocated: None in FY 1972

The primary objective of this contract is to determine whether functional stages of brain development can be identified, described, and quantified by means of mathematical analytic techniques. Time series analysis methods are being applied to electrophysiological signals collected from children. Pattern recognition procedures will then be applied to the descriptors derived from the time series analyses. In addition, efforts are being directed toward determining the sensitivity of mathematical procedures in discriminating among special populations, e.g., the impact of an impoverished environment on brain development, identification of children with learning disorders, and identification of human genetic variations in fraternal and identical twins.

This contract will be terminated on June 30, 1972. Final reports are in preparation. Under NICHD auspices a most singular and unique laboratory has been created for special quantitative analyses of neurolectric activity that is currently not available in other laboratories. The most significant contribution of this laboratory has been the development of mathematical computer techniques to detect deep brain spiking activity from surface scalp EEG recordings. When perfected for use with patients, this capability will expand clinical diagnostic procedures and make it possible to screen for brain dysfunctions not currently easily diagnosed.

Growth and Development Branch
Contract and Collaborative Research

Contract Number:	N22 22737
Contract Title:	Analysis of Data from an Ecological Study of Infection, Malnutrition, and Growth of Children in a Guatemalan Indian Village
Contractor:	Pan American Health Organization, Institute for Nutrition in Central America and Panama Leonardo J. Mata, D.Sc.
Money Allocated:	$73,000 (U.S.-Japan Cooperative Medical Science Program Funds) FY 1972

The primary objective of this contract will be the analysis and publication of the data from a longitudinal study of nutrition, infection, and growth among pregnant and lactating women, infants, and young children in a small native village in Guatemala. Throughout the study attempts were made to interfere only minimally with the natural ecosystem of the village and the family. Approximately 45-50 babies have been born per year in this village, providing a population in excess of 400 newborns over the eight year study period (1964-1971). Growth and morbidity data have been obtained on all children. More detailed studies on neonatal infections, their nature, and origin have been done on 350 children. Within this latter group, 45 (representing 50% of those born during 1965-1966) were identified for intensive study from the prenatal period through the first seven years of life. Regular data were obtained on these children concerning height, weight, days of illness, dietary intake and nutritional status, and infections and colonization of the intestine as indicated by weekly stool samples. Twelve of these had more intensive work-up of patterns of development of indigenous microflora.

Data analyses will be done using special programs available at the University of Washington, Seattle, to supplement analyses at the Principal Investigator's facility (INCAP). Some viral identification also will be completed to round out the data base. It is anticipated that no more than two years will be necessary to complete the objectives of the contract.

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Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01 12420
Contract Title: Research Materials and Assistance for Studies of Language Development in Children
Contractor: Haskins Laboratories, New Haven, Connecticut
Franklin S. Cooper, Ph.D.
Money Allocated: None in FY 1972

The objective of this contract is to provide stimulus materials and specialized research assistance to selected investigators, hereafter known as user groups, in the area of language studies in children. During the first year this has usually involved indoctrination into the use of the Haskins Laboratories computer-based speech synthesis system, followed by extensive use of that system by the user group in preparing tape recorded stimuli in synthetic or natural form, and for monaural or binaural or dichotic presentation. Working visits by users have also usually involved extensive discussions of the proposed research with members of the Laboratories' scientific staff.

During the initial nine months of the contract, requests for assistance were received from 21 users affiliated with 15 leading universities. Thirteen of the users were faculty members and eight were graduate students; for most of the latter, the requests came from the students' thesis advisors. All of the requests were acceptable by the criteria for relevance and scientific merit described in the contract; many appeared to be excellent. Three of the requests are still in the planning stage; for the other 18, users have completed the preparation of one or more sets of stimuli during a total of 95 days spent at the Laboratories. More than half of this latter group of users groups have plans to return at a later time to prepare additional sets of stimuli.

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Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01 71451
Contract Title: Developmental Electrophysiology of Brain Function and Behavior
Contractor: University of Utah, Salt Lake City, Utah
Edward C. Beck, Ph.D. and Robert E. Dustman, Ph.D.
Money Allocated: None in FY 1972

The objectives of this contract have been to establish basic data on cortical evoked potentials (CEPs) from the visual, auditory, and somatosensory modalities on normal children age 4-16; to conduct studies on the effects of impoverished environments on children's brain function, as measured by CEPs, and to conduct studies on identical twins.

Although no FY 1970 or 1971 funds have been allocated to this contract the principal investigators have continued to analyze and report upon the large volume of data collected during the contract project period. One of the most significant findings and contributions has continued to be the demonstration of a relationship between brain function asymmetry and intellective-cognitive functioning. In particular, it has been found that impoverished families consistently on welfare compared to impoverished families not on welfare have a higher proportion of offspring whose brain functioning is characterized by brain asymmetry similar to that seen in more intelligent pairs of non-impoverished children. What aspect of services provided to welfare families may relate to the observed differences in brain electrophysiology is not clear from these studies.

This contract will be terminated June 30, 1972. Final reports are in preparation. A recent staff site visit has confirmed that all contract objectives have been met and that this research program has made significant contributions to developmental neuropsychology.

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Growth and Development Branch
Contract and Collaborative Research

Contract Number: N01 50640
Contract Title: Study of Relationship Between Nutrition and Behavioral Development
Contractor: Pan American Health Organization, Institute of Nutrition for Central America and Panama
Robert Klein, Ph.D.
Money Allocated: $563,757 (FY 1972)

This contract has been designed to contribute additional knowledge of the relationship between nutritional status and physical, psychological, and social growth. Two pairs of small villages (aldeas) of approximately 650 inhabitants each have been matched on a variety of sociocultural variables. In one village of each pair a dietary supplementation program is provided with special effort made to include in this supplementation program every preschool child and pregnant woman in the village. Incaparina, a high protein food supplement, is made available to every person in the village who wants it. In the control villages medical service is offered, but no food supplementation is provided. Thus a sample of well-nourished preschool age children can be compared on relevant dimensions with a sample of medically treated preschool children raised under the dietary conditions characteristic of the area. Children will be followed from birth until about six years of age, at which time effects of differences in nutrition should be clear.

The long-term study has now been underway for two years. Special emphasis has been placed upon assuring supplementation of pregnant women in the study villages. Preliminary data suggests that this supplementation effort has significantly increased the birth weight of infants born into the study. However, this conclusion cannot be considered firm until after all infants have been enrolled from the first three years of intake. The overall field operations have been highly successful. Data collection is continuing at a satisfactory rate. The staff of the project are now outlining detailed methods for analyzing the data as it is accumulated and at the completion of the proposed longitudinal study.

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Perinatal Biology and Infant Mortality Branch

The objective of the Perinatal Biology and Infant Mortality Branch is the promotion of a coordinated program of research and training which will enhance understanding and development of knowledge as related to pregnancy maintenance and management, embryonic development, fetal growth, and infant well being through the first year of life. Efforts are also directed toward reducing this country's infant mortality rate, ameliorating infant morbidity, and narrowing the gap between the identification of new knowledge and its incorporation into the delivery of health care.

Program goals recognize the interrelationships of specific health and developmental problems encompassed in the prenatal, perinatal, and infant period of life, and the effects that these events may have upon subsequent development and well being of the child. Particularly relevant are those morbidity- and mortality-related maternal health problems which affect fetal and infant health status, problems with which the newly born infant must cope in his adaptation to extrauterine life and subsequent survival and well being, and events occurring during the period of hospitalization following birth which can influence the subsequent behavior and development of the baby.

The Branch supports research and training concerned with various aspects of the physiology of pregnancy, disorders of pregnancy, placental function, immunologic phenomena, developmental biology, and physiology, and the biochemical processes of embryonic and fetal growth, the process of labor and delivery, including biophysical and biochemical diagnostic procedures; and genetic, pharmacologic, toxic, and infectious processes and influences which can effect the mother and fetus. Primary attention in the area of infant survival and well being emphasizes respiratory function, metabolic processes, immunology and blood dyscrasias, infectious diseases, congenital malformations, and sudden unexpected death.

The Perinatal Biology and Infant Mortality Branch is responsible for the supervision and administration of approximately 23 percent of the Institute's extramural holdings, totaling about 267 grants and contracts and amounting to over 12.3 million dollars. The Branch is organized into four broad program areas: Pregnancy and Maternal Health, Developmental Biology, Fetal Health and Development, and Infant Survival and Well Being. The first three program areas are each under the supervision of a Health Scientist Administrator responsible for the administration of research grants, training grants, career awards, and fellowships comprising the subprogram content. Currently, no one professional person is responsible for the Infant Survival and Well Being Section; related responsibilities are shared by all staff.

The total program holdings assigned according to program sections are shown in the following table:

(in thousands of dollars)

Mechanism of Support	Total		Pregnancy & Maternal Health		Developmental Biology		Fetal Health and Development		Infant Survival and Well Being	
	No.	Amount	No.	Amount	No.	Amount	No.	Amount	No.	Amount
Total	267	$12,344	70	$2,954	64	$4,103,	69	$2.512	64	$2,775,
Research Grants	192	8,966,	59	2,642,	40	2,333,	48	1,972,	45	2,019,
Training Grants	27	2,165,	4	137,	16	1,656,	5	228,	2	144,
Fellowships	19	199,	2	19,	5	51,	5	53,	7	76,
RCP Awards	25	626,	4	115,	3	64,	11	259,	7	188,
Contracts	4	388,	1	40,	-	-	-	-	3	348,

Note: This excludes one research grant for $55,498 paid from Special Genetics Funds of NIGMS.
Dollar figures may not add to totals due to rounding.

Approximately one quarter of the grant holdings are concerned with training support. Sixteen of the training programs provide basic science research training in the field of developmental biology, six of which are closely allied to clinical departments. One training program is in a school of veterinary medicine. The remaining ten programs are associated with clinical medicine and are conducted within departments of obstetrics and pediatrics. A serious gap exists in the PBIM training program in preparing research neonatologists; of the ten training programs in clinical medicine, only two are preparing trainees in the area of neonatal biology.

Areas of scientific responsibility and interest for each of the four program areas are highlighted in the following paragraphs; selected scientific accomplishments are included.

Pregnancy and Maternal Health

The Pregnancy and Maternal Health area of the Branch is concerned with normal and abnormal physiologic events and changes associated with the gravida state during the antepartum, intrapartum, and puerperium periods. To more fully understand the underlying mechanisms pertaining to normal maternal events, information is sought about the role of the endocrine glands, respiratory tract, cardiovascular system and genitourinary tract in pregnancy and their involvement in abnormal conditions and disorders such as diabetes, toxemia, hypertension, isoimmunization, malnutrition, viral and bacterial infections, anemia and blood dyscrasias, and other hemorragic phenomena associated with pregnancy. Of significant interest to the program is research concerning functions of the placenta, psychosocial dynamics of pregnancy, factors involved in the maintenance of pregnancy and the initiation of labor as well as an appreciation of intrauterine environmental hazards caused by drugs, chemicals, anesthetics, or common pollutants.

Significant scientific accomplishments have been achieved as a result of NICHD grant support; these are highlighted in the following paragraphs.

Advances are being made with regard to the endocrinology of pregnancy. A research career development awardee, Dr. John Reynolds of the University of Minnesota together with other researchers, recently defined alternative and possibly adaptive mechanisms by which steroids are metabolized in the developing fetal-placental unit. They have discovered that steroid metabolism does not proceed in the same way during pregnancy as in non-pregnant states, and have defined how the pathways differ. This work has possible significance in that this may be the means by which a fetus is protected from toxic steroid levels in utero; it may be the pathway by which the fetus signals that it is ready for extrauterine adaptation, or it may be the means by which the enzymatic induction necessary for extrauterine survival could be clinically manipulated.

Clinical research having to do with steroid biochemistry is resulting in interesting findings. Dr. Mortimer Levitz of New York University, another research career development awardee, has completed initial work which characterizes maternal steroid (estriol and cortisol) profiles specific for a variety of maternal pathological states. This work should greatly aid clinicians in deciding when intervention is optimal in order to maximize fetal salvage in conditions such as preeclampsia and diabetes.

Dr. Valerie Ann Galton of Dartmouth Medical School using recently developed radioimmunoassay methods, has shown that subclinical maternal hypothyroidism may be induced by estrogens and pregnancy. This work has also tentatively defined harmful effects of the hypothyroid state on the developing central nervous system. Once these effects are fully characterized, the administration of thyroxine to the pregnant woman may overcome detrimental effects on the fetus caused by subclinical maternal hypothyroidism.

Dr. Edward Kass and his co-workers of Harvard University are continuing to refine their studies concerned with infectious processes and pregnancy. They have found that genital mycoplasms are a factor in the genesis of pregnancy.

wastage. The extent and significance of this factor has not been completely defined, and merits the further investigation now under way. They have also shown that bacteruria in pregnancy is not an isolated phenomenon. Bacteruria continues to persist in a significant number of females for periods up to 14 years. Since this continued bacteruria is associated with decreased renal function, IVP abnormalities, and the development of hypertension, the serious public health implications of these findings are apparent.

During the 1970 NICHD conference concerned with the initiation of labor, investigators pointed out that one area of top priority for future research concerned factors that control uterine muscle membrane excitability. At Duke University, Drs. Anderson, Ramon, and Snyder have conducted research which defines membrane excitability in terms of the NA^{+} and CA^{++} permeability system. Further work of this type is expected to lead to control of membrane excitability and therefore prevent premature labor. Since prematurity accounts for approximately two-thirds of all perinatal deaths in this country, research in this area has high priority.

The role of maternal folate deficiency in the genesis of maternal hypertension, abruptio placentae, fetal malformations, and abortions has continued to be questioned by the obstetrical community. Recent research completed by Dr. Jack A. Pritchard at the University of Texas Southwestern Medical School has demonstrated that maternal folate deficiency plays no role in the etiology of these conditions.

Developmental Biology

The Developmental Biology program area is concerned with fundamental research pertinent to the understanding of human perinatal biology. Knowledge of molecular and cellular biology is integrated at increasing levels of developmental organization by the support of research on systems extending from single celled animals to the more biologically complex systems including primates. Emphasis is placed not only on investigations with mammals, but on other model systems which afford distinct advantages for the study of development from conception through embryogenesis and into the first year of life.

Major areas of research currently supported include the genomic regulation of development, the development of the immune system, developmental endocrinology, organization of the cell, environmental impacts on development, pattern formation and morphogenesis. Recent research emphasis on pattern formation and morphogenesis by PBIM grantees has further clarified the nature of cell surface components, the role of microtubules in determination of cell shape, inductive tissue interactions and gene control of development. Scientific accomplishements by investigators whose research is supported by the Developmental Biology program areas follow:

Cellular recognition in vertebrates has achieved great sophistication, reaching an advanced stage in mammalian systems. A recent achievement in understanding the essential role of like to like recognition in developmental process has been made by Dr. Aron Moscona and colleagues at the University

of Chicago. These investigators stripped embryonic mouse cerebrum cells of surface components by a wash procedure and obtained a cell binding ligand factor from the dispersal of cells which when added to cultures of cerebrum cells causes them to aggregate into large clusters. Discovery of this specific cell ligand represents a first isolation of a cell binding material from mammalian cells; previously ligands have been obtained from sponges and avian retina. The question remains as to the role of such a ligand in the sorting out processes used in development in vivo; further work will be required to determine whether these properties are a consequential rather than casual mechanism in embryogenesis. Biochemical and immunological characterizations of the ligand in the near future should bring about discussion of how different kinds of cells of an embryo recognize and assemble into complex systems in much simpler terms. Greater understanding of how congenital growth defects occur and may be prevented should be forthcoming.

In morphogenesis of salivary gland epithelium, it is clear that extracellular materials are essential if epithelial morphogenesis is to occur. Nevertheless, as revealed in experiments by Dr. Brian Spooner of Stanford University, it is the system of microfilaments within the epithelial cells that is the key to that phenomenon. If salivary glands from 13-day mouse embryos are treated with cytochalasin in organ culture, striking changes occur. The epithelium flattens into a thin, wafer-like sheet and loses the clefts or branch points formed during earlier morphogenesis. This epithelium remains quiescent and does not continue morphogenesis until the drug is removed. These studies add to an understanding of the normal development and function of tissues covering internal and external body surfaces during embryogenesis.

In most tissue interaction studies undertaken in early developmental periods, it is assumed when the tissue does not differentiate after isolation, that the experimental situation is representative of in vivo systems. Studies by Dr. James Lash and co-workers of the University of Pennsylvania are important in indicating that most tissues have a greater differentiative bias than previously thought and should lead to clarification of the nature of inductive tissue interactions, clearly of major importance in early embryogenesis. These investigators found that while it is possible to demonstrate an effective interaction in which embryonic chick notochord promotes the differentiation of cartilage from somitic tissue, the negative result when somites alone fail to undergo chondrogenesis, applies only to a prescribed set of culture conditions. By substituting fetal calf serum and other nutrient medium, the incidence of in vitro chondrogensis is markedly enhanced in somites cultured in the absence of notochord. The notochord, therefore, does not impose chondrogenic information upon the somites; it only permits or enhances a preexisting chondrogenic bias of the somites.

Certain nonmammalian systems offer special advantages for the dissection of complex developmental phenomena at the level of specific genes. Questions about limb formation and normal development intractable in other systems can be explored. Taking advantage of the unique opportunities offered by the phenomena of somatic pairing exhibited by the chromosomes of Drosophila, Dr. P. J. Bryant of the University of California at Irvine has induced by

x-ray treatment somatic recombination and change in the genotype of cells at various stages of development. This technique enables the investigator to determine the career of individual cells and their descendants with great precision and to analyze developmental processes in far greater detail than is possible in other systems. From experiments in which the legs were transected Dr. Bryant has provided evidence for an actual development gradient with regulatory functions. The upper part of the limb tends to develop normally, the lower half to duplicate with mirror symmetry. Some positional values for specifying the limb structure have only occurred in the original top portion of the gradient whereas those in the relaxed lower portion are duplicated.

Fetal Health and Development

The fetal health and development area is concerned with a wide range of processes and problems relating to fetal growth and development at tissue and organ levels. Research support aims to further our understanding of normal embryonic development and to clarify the causes, mechanisms, prevention, pathophysiology and treatment of specific events detrimental to the fetus. Emphasis is given to the interrelationship of pharmacological, nutritional, immunological, metabolic and other physiological factors which are important to fetal health and growth from implanation until parturition. Basic genetic, biochemical, and biophysical investigations are considered essential, not only as a basis for fetal status evaluation, but also for meaningful antenatal diagnosis. In order to better understand the environmental conditions which affect the welfare of the fetus, studies are supported on the functional role of the placenta involving feto-maternal gas exchange, placental transfer of nutrients and drugs, and feto-placental hemodynamics. Efforts are also directed at research elucidating the effects of maternal nutrition and diet, hormones, infections, drugs, and smoking on the developing fetus.

Selective research accomplishments for the past fiscal year are highlighted in the following paragraphs:

In recent years, much emphasis has been placed on the biochemistry, enzymology endocrinology, and immunology of the developing placenta. This work has been accomplished without sufficient concomitant studies of placental ultrastructure and morphology. This gap in knowledge presents problems in that any future attempts to repair deficient, or maximize otherwise normal placental function, must be undertaken at the sub-organ or cellular level if success is to be realized. This will be impossible if the precise morphological correlates of function remain undefined.

Recent studies by Dr. Ralph Wynn of the University of Illinois have begun to close this knowledge gap. Working with modern electron microscopic and other physiologic techniques, Dr. Wynn and his research team have begun morphological correlation of specific placental function. Studies conducted in the baboon detailing the ultrastructural changes of the endometrium, vagina, ovary, and placenta allow this animal to be used as a model for the study of all phases of reproductive biology. Studies in the hamster have

begun to define the mechanism whereby initial support of the fetus by the uterus is accomplished and maintained. Similar studies in the human have delineated specific cellular sites that produce the functional changes in the placenta required by the fetus for survival. Now that cell type specificity is documented, it is assumed that future research will address itself to experimental manipulation of the variables involved, in order that maximal fetal well being will be achieved under a variety of abnormal conditions.

The unique role of the human placenta in the maintenance of normal fetal development and in its ability to act and interact with exogenously administered drugs and chemicals reveals its remarkable multiple functions. Since mixed-function oxidation reactions are extremely important in the metabolism of both drugs and steroids, the necessity to study placental tissue is obvious. Dr. M. R. Jucheau of the University of Washington investigated the effect of cigarette smoking on human placental benzpyrene hydroxylase (BPOHase) during early gestation and explored various characteristics of the human hydroxylase system. It was discovered that human placentas obtained from women who smoke possess significant capacity to catalyze the hydroxylation of BP, a carcinogenic hydrocarbon present in tabacco smoke. Further studies revealed that this placental hydroxylating capacity did not exist to a significant extent during the first trimester of pregnancy. This finding is of importance since it is well documented that many drugs produce teratogenic effects during the first trimester; a lack of significant placental drug metabolizing activity during this period could present an additional danger to the fetus, particularly if a teratogen or mutagen were active in the unmetabolized form. The findings of this investigator are of further significance because it has been speculated that during early gestation the placenta may serve as a fetal liver until the fetal liver can develop its own hepatic functions. Dr. Jucheau's results indicate the converse may be true with respect to placental drug metabolic activity; hydroxylase activity was relatively high at term but not readily detectable during early gestation.

Studies of fetal nutritional requirements and the production of neonatal mortality and morbidity continue to receive high priority from scientists supported by the Institute. Dr. Fred Battaglia of the University of Colorado using the fetal sheep as a model has begun to characterize specific types of nutrients necessary for fetal development, as well as the conditions under which these nutrients are optimally utilized. Studies of specific organs (e.g., brain, liver, heart) and the selective use of nutrients by these organs have also been completed. Dr. Battaglia's data suggest that the fetus does not depend on glucose in the usual manner as previously assumed; moreover, different organs utilize different nutrients at various stages of gestation. This work assumes increasing importance as it becomes easier to diagnose fetal undergrowth in utero. Through the studies of Dr. Battaglia, it can be assumed that rational replacement nutrient therapy could be achieved while allowing the fetus to remain in utero. Significant behavorial data collected by Dr. B. F. Chow of Johns Hopkins University also emphasizes the importance of nutritional studies. This investigator, using the rat as an experimental model suggests that the fetus, by cumulative effects of maternal malnourishment, can be so affected as to sustain behavioral abnormalities of long duration.

The teratogenic effects of nutritional deficiencies have been produced both by long-term chronic depletion of a specific nutrient or by acute metabolic deficiency by an antimetabolite. Reports on the congenital malformations of surviving fetuses born of zinc deficient female rats suggest that the effect of shorter periods of zinc deprivation may be more critical than the long-term chronic deficiency state. Dr. L. S. Hurley of the University of California investigated short-term and transitory zinc deficiency in rats by feeding female rats a zinc-deficient diet during selective times in pregnancy. Results indicate that while short-term zinc deficiency had no appreciable effect on the maternal organism, impaired reproduction and a high incidence of congenitally malformed young occurred even though a normal diet adequate in zinc had been fed until the beginning of gestation. Transitory periods of deficiency altered the incidence of the anomalies observed in accordance with the developmental events occurring at the time the deficiency was imposed. The rapid and severe effects of relatively short periods of zinc deficiency on fetal development suggest that the pregnant rat cannot mobilize zinc from body stores in amounts sufficient to supply the needs of developing fetuses. The significance of these observations to the human must be investigated further.

Infant Survival and Well Being

This program area is concerned with basic and clinical studies of the etiology, pathophysiology, therapy, and follow-up of conditions and syndromes associated with the postnatal period up to one year of age, such as asphyxia, respiratory distress, hypoglycemia, hyperbilirubinemia, anemia, erythroblastosis fetalis, sudden infant death syndrome, etc. Of equal importance are the physiological, biochemical, and behavioral aspects of extrauterine adaptation, including respiratory, cardiovascular, thermodynamic, hematologic, and metabolic changes associated with adjustment to the extrauterine environment.

Interests in this area also include studies of the influence of maternal and environmental conditions, events, and treatments as they affect neonatal adaptation and the elucidation of optimal nutritional, environmental, and metabolic requirements for both normal and high-risk infants in the transition to an extrauterine existence. There is also major interest in investigations concerned with the effects of those events or therapies occurring during the early days of life upon adaptation and subsequent development, particularly as they involve the high-risk newborn and relate to such conditions as artificial ventilation, acidosis, hypoglycemia, hyperbilirubinemia, phototherapy, and minimal handling. The etiology, pathophysiology and management of specific congenital malformations observed during the newborn period are of concern to this program area. Of significant importance are investigations concerned with the etiology, epidemiology, and underlying mechanisms of the sudden infant death syndrome, identification of infants at high risk to become a sudden infant death victim, and development of preventive measures. Several interesting findings resulting from grants supported in this section are highlighted in the following paragraphs.

A significant research effort directed at the fetus in utero will probably lead to major reductions in the rate of development of postnatal problems.

Through the efforts of Dr. Louis Gluck of the University of California at San Diego, it is now possible to assess prospectively by analyses of the lecithin and sphingomyelin content of amniotic fluid whether or not fetal lungs are mature enough to withstand extrauterine existence without developing hyaline membrane disease. Because of this, it is now possible to know whether delivery intervention with a given pregnancy is warranted.

The therapy of respiratory distress syndrome has been significantly enhanced through the efforts of Drs. William Tooley and Rodney Phibbs at the University of California in San Francisco, Dr. Alan Hodson at the University of Washington, Dr. Thomas Oliver at the University of Pittsburgh, and Dr. Peter Auld at Cornell University. Their independent research has pointed to the use of the more "physiologically sound" continuous positive airway pressure (CPAP) instead of the traditional intermittent positive airway pressure (IPAP) in artifically ventilating sick neonates. Early work would seem to indicate that CPAP has the advantage over IPAP in that it prevents air trapping in the alveoli and favorably alters ventilation perfusion. Initial results have been very encouraging, and further confirmation of this work is awaited.

Through the work of Dr. U. Muller-Ebhard of the Scripps Clinic and Research Foundation, Dr. Gerold O'Dell of Johns Hopkins University, Dr. Michael Thaler of the University of California, and Dr. Jerold Lucey at the University of Vermont the increasingly complex problem of hemolytic disease of the newborn may yield to a new treatment regime which may make therapy a simple process. Excess bilirubin accumulation in the newborn can no longer be viewed as simply excess hemolysis coupled with immaturity of the glucuronidase enzyme system. It is now known that the enteric circulation of bilirubin (as modified by the gut bacteria), binding proteins in the liver, genetic inheritance, and the amount and qualitative variation of albumin produced by the neonate all influence the clinical course of this syndrome. Better cellular and biochemical markers for risk of kernicterus have been developed, thereby allowing the clinician to propose exchange transfusion in a more rational fashion. The use of light as a therapeutic alternative for a majority of exchange transfusions has been established. Further work on the indications for light treatment, as well as its long term effects on the developing infant are needed in order that the cure does prove to be worse than the disease.

From the investigations of Dr. E. Kass of Harvard University, Dr. C. Alford of the University of Alabama, and Dr. D. Lang of Duke University have come data suggesting a new area of research concerned with infection and development. Dr. Kass has demonstrated that asymptomatic mycoplasm infection in pregnant women leads to a significant decrease in birthweight. The current work of Drs. Alford and Lang have expanded previous work on cytomegalic and other non-clinical infectious illnesses of pregnancy to include not only diagnostic methodology, but inquiries into the pathogenesis of <u>in utero</u> infection. That systemic immune alteration can persist in the developing infant with associated mental morbidity is well established. These workers are currently proposing non-clinically manifested maternal antigenic overload with subsequent fetal damage as a reasonable explanation for low birthweight and subsequent central nervous system morbidity. The pathophysiology of such a hypothesis, which has grown out of their current research, will be of top research priority for these investigators in the future.

Follow up of mothers and infants who have been considered high risk in the perinatal period is an integral part of program objectives and goals. It is ssential that the sequelae of high risk perinatal conditions be identified if we are to fully understand the significance of various events that take place in the perinatal period and to appreciate their impact both on maternal health and subsequent growth and development of the infant.

Dr. Jerold Lucy at the University of Vermont is currently evaluating the physical, neurologic, and psychometric status of babies who had been treated with phototherapy between 1966 and 1968. Dr. Charles Alford of the University of Alabama is undertaking microbiologic and immunologic follow up of development of infants who had been victims of "chronic" intrauterine viral infections. Evaluation includes microbiologic, immunologic, growth, motor, sensory, perceptual and social development assessment. These studies will clarify the medical significance of congenitally acquired infections since most infected infants appear normal at birth.

Dr. William Oh of the University of California recently completed neurologic, psychometric and electroencephalographic follow up examinations between twelve and fifty-four months of age on ten infants surviving from severe Rh erythroblastosis fetalis which required intrauterine blood transfusions. He found no differences in these ten infants for the variables tested as compared to matched controls. These results appear to suggest that severe Rh erythroblastotic infants who receive intrauterine blood transufsions have a normal developmental performance at a later age.

At the University of Colorado, Dr. Lulu Lubchenco's meticulous clinical observation and long term follow up studies have defined high and low risk in the neonate by a combination of both gestational age and weight. This has led to a gradient of risk being assigned to each neonate with subsequent placement of newborn infants on the basis of risk to special personnel and facilities. Furthermore, long term follow up has shown certain groups to be at greater risk than others for morbidity; in the future, rehabilitative efforts can be more rationally programmed. For example, infants with birth weights of 1001-1500 grams have a 50% mortality rate if their gestational ages are less than 34 weeks, but only a 13% mortality rate if their gestational ages are 34-40 weeks. Follow up examinations are underway for both small for gestational age and appropriate for gestational age infants who were studied for hypoglycemia in the newborn period.

During the past year, increased emphasis has been placed on expanding program activities associated with the sudden infant death syndrome particularly as related to its cause. In early July 1971, the Program Director and a representative from the Office of Planning and Evaluation addressed the participants of the First National Parent-Medical Conference on Sudden Infant Death Syndrome concerning Federal activities in this area. In mid-August 1971, the Branch organized a program planning workshop for the purpose of identifying the most fruitful hypotheses to be explored relevant to underlying mechanisms of the syndrome. Representatives from fields of neurophysiology, cardiorespiratory physiology, immunology, microbiology, veterinary medicine,

pathology, and epidemiology participated. This workshop provided a foundation for expanded program activities in sudden infant death syndrome. As a result of discussion and interaction, it became evident that the most fruitful approach evolved around multifactorial hypotheses relevant to perinatal maturation involving the physiology of sleep, cardiorespiratory function, and mechanisms of immune response particularly as these pertain to infection.

Staff has developed an annotated bibliography of the SIDS literature from 1960-1971; and prepared for publication the booklet Summary of Proceedings: Second International Conference on Causes of Sudden Infant Death Syndrome and a brochure entitled Facts About Sudden Infant Death Syndrome. The Branch has worked with the National Center for Health Statistics for the inclusion of a specific category for sudden infant death syndrome in the Ninth International Classification of Diseases. The Branch has also established liaison relationships with the Maternal and Child Health Program of the Health Service and Mental Health Administration on this problem.

Dr. C. George Ray and associates of the University of Washington continue their studies on the role of viruses in the sudden infant death syndrome. Coupled with previous studies, their data suggest that viral infection, if it participates in the ultimate mechanism in SIDS, does not act by means of overwhelming viremia, but rather may act locally, possibly in the respiratory tract. Dr. Marie Valdes-Dapena continues her research on the role of the conduction system as a possible cause of crib death. Her work to date shows no significant evidence of pathologic change in the conduction system of SIDS victims. The islands of pallor show neither degeneration nor necrosis; the cells composing these areas are found to be healthy and the fibrils intact. During the fiscal year, the Institute has awarded a research grant to Dr. Peter Katona of Case Western Reserve University for study of normal development of autonomic reflexes during the first year of life in premature and full term infants. Reflex sensitivity will be quantitated, and the hypothesis that infants who die of SIDS have higher reflex sensitivity than a control group of infants will be tested.

Conference and Publications

In addition to previously mentioned SIDS Conferences and Workshops, the program sponsored three interdisciplinary conferences: "Sensory Needs of the High Risk Newborn and His Parents" (July 1971); "Intravenous Nutrition for High Risk Newborns" (October 1971); and "Narcotics, Pregnant Women and Their Children" (May 1971). Publication of the proceedings of each conference is underway or planned. Two conferences have also been supported through the research grant mechanism: "Perinatal Development" and "Workshop in Teratology as Related to Toxicology." Edited proceedings of the second conference are available.

The Program is currently supporting the preparation of a catalog of teratogenic agents to facilitate dissemination of scientific data on the rapidly accumulating number of compounds to which the human embryo can be at risk. Approximately 1,000 compounds reported to cause teratogenic abnormalities in man and animals will be listed along with information on species studied, dosage, time of

administration, defect observed, and key references. The catalog will serve obstetricians and pediatricians directly concerned with the prevention and treatment of the major health problem posed by congenital defects in children, as well as pharmacologists, experimental teratologists, environmentalists and others in the biomedical community seeking to understand and alleviate the action of teratogenic compounds on human development. Preparation of this catalog is under the direction of an NICHD consultant who is a pediatrician with extensive experience in human embryology and teratology.

Foreign Country Activities

PL 480 Program: The PBIM Program currently administers two PL 480 research projects both in Yugoslavia (Drs. N. Skreb and S. Milkovic). In the Fall 1971, a PBIM staff member visited Poland and Yugoslavia in order to explore and develop additional collaborative PL 480 research proejects relevant to perinatal biology and maternal and infant health. Of the eighteen research proposal drafts presented by the host countries, twelve were selected for further review and consideration at the site; additional proposals have been received since the visit. Currently thirteen proposals are in various stages of review. As a result of the enthusiasm evidenced by scientists in Yugoslavia and Poland, continued efforts will be made to explore additional PL 480 collaborative research projects subject to availability of funds.

INSERM: The PBIM Program has been participating in the NIH collaborative research activities and exchange program with the Institute National de la Sante et de la Recherche Medicale (INSERM) in the area of perinatology under the coordination of the Fogarty International Center. The PBIM United States coordinator is Dr. Norman Kretchmer, Professor of Pediatrics and Human Development at Stanford University. During the past year one scientist, Dr. Jeanne Roux from Professor Alex Minkowski's laboratory in Paris, spent three months in Dr. Kretchmer's laboratory and currently Dr. Nicholas Hoogenrood, an investigator in Dr. Kretchmer's laboratory, is working in Dr. Minkowski's laboratory. An exchange of additional scientists is anticipated during the next year.

Personnel

The PBIM Program is assigned four professional positions; one secretarial position, one clerk stenographic position, one clerk typist position, and a grant's assistant position. Three of the professional positions are staffed with persons holding Ph.D. degrees. The other has an M.D. The program is also assisted on a part-time basis by a professional staff person assigned to the Planning and Evaluation Program who serves as Executive Secretary to the PBIM Research and Training Committee and assists in handling other program activities.

In FY 72, two NIH Grants Associates worked in the Branch. A nine week work experience in health science administration was provided for two second year students from the Medical College of Pennsylvania as part of their PHS COSTEP experience. One of their projects was the development of a classification system for the Infant Survival and Well Being program section; the other was the compilation of the SIDS Annotated Bibliography previously mentioned.

The Branch has actively participated in the NIH Upward Mobility Program. A summer aid and two stay-in-school students have worked in the program for time periods ranging from ten weeks to four months. Two secretaries are currently enrolled in the NIH Federal City College Program. A cash award was presented to a clerk typist in recognition for guidance given to the summer aid and stay-in-school students and for outstanding work performance in assisting the COSTEP students in the preparation of the Sudden Infant Death Annotated Bibliography.

NICHD Annual Report
July 1, 1971 through June 30, 1972
Perinatal Biology and Infant Mortality Branch
Contract and Collaborative Research

Contract Title : Cooperative Hospital Study on the Respiratory Distress Syndrome in Infancy
Contractor : Case Western Reserve University
Money Allocated : $21,100

Objectives: To undertake a collaborative study directed at comparing two regimens of early treatment of respiratory distress syndrome which differ only in the concentration of oxygen (80% versus 40%) in the environment beginning at one hour of age until six hours of age.

Significance to Biomedical Research and Program of the Institute: Idiopathic respiratory distress syndrome represents a major health problem throughout the world. In the United States of America, approximately twenty-five percent of infants born prematurely show signs of this disease within the first 24 hours of life. Twenty to forty percent of these babies succumb from it, accounting for about 25,000 infant deaths annually in this country.

Empiricism, rather than controlled clinical trials, have led to a variety of practices in the management of various aspects of the neonatal respiratory distress syndrome without any marked changes in mortality. It is now recognized that pulmonary hypoperfusion is an important factor in the pathogenesis of the respiratory distress syndrome, and that therapy should be directed to correction of pulmonary arterial flow. Methods which have been shown to be of value in normalizing pulmonary vascular perfusion are: the reduction of hydrogen ion concentration, increased oxygenation, and mechanical inflation of the lung. Because oxygen and pH can be more easily controlled, it is important to evaluate their effects before embarking on a study of mechanical ventilation. It is clear, however, from the findings of many investigators that correction of acidemia is imperative. There is controversy, however, with respect to the use of environmental oxygen supplementation because of the dangers of retrolental fibroplasia. To prevent retinal damage, the arterial oxygen concentration must be kept below the level that stimulates vasoconstriction. The arterial blood oxygen is crucial, not the ambient concentration. Thus, there is a need for a valid statistical design to evaluate the effects of oxygen therapy with careful monitoring of arterial oxygen tension to prevent the development of retrolental fibroplasia.

Proposed Course: Details of the protocol have been designed to provide high-grade intensive care from the earliest possible time; no experimental techniques or medications have been introduced. Each subject admitted to the study must weigh 1,000 grams or more at birth; be breathing without assistance by the age of ten minutes; by sixty minutes of age more than 50% of his respiratory activity must be accompanied by grunting, whinning or a complaining cry or groan on expiration; be free of any major congenital anomalies which could contribute to respiratory distress, be no older than

one hour and fifteen minutes of age. Once a patient is admitted to the study, the assignment to one of the treatment regimens is made without bias, so that resulting data will show the relative merit of the two forms of early management.

Arterial blood oxygen concentrations are monitored through umbilical catheterization during the five hours following admission to the study. At six hours, the infant's clinical condition and laboratory values are evaluated using the scoring system developed for this study. This scoring system is recorded at 1, 6, 12, 24, 48, and 72 hours. All infants after six hours are treated according to the same regimen.

All data are submitted to the Department of Biometry at Case Western Reserve University for statistical evaluation.

Admission of subjects to the study ended on March 31, 1972. As of this date, a total of 151 subjects were admitted and retained in the study. It is planned that the work during the next year will be devoted to intensive analysis of all data collected.

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July 1, 1971 through June 30, 1972
Perinatal Biology and Infant Mortality Branch
Contract and Collaborative Research

Contract Title : Intrapartum Fetal Monitoring
Contractor : Professional Staff Association of the Los Angeles County/University of Southern California Medical Center, Los Angeles, California
Money Allocated : (No funds committed)

Objectives: The objective of this project is to characterize the changes and patterns associated with specific groups of high-risk obstetrical patients through detailed study and analyses of fetal heart rates (FHR), fetal electrocardiograms (FECG), uterine contractions (UC), and fetal acid-base status and respiratory gases. This work should help to clarify the value and merits of biochemical and biophysical methods of fetal surveillance in specific groups of high-risk obstetrical conditions. The high-risk obstetrical conditions to be studied include toxemia, chronic hypertension, Rh sensitization, abruptio placentae, maternal diabetes, premature rupture of membranes, premature labor, prolonged pregnancy, para 6 or greater, elderly primigravida, breech presentation, previous cesarean section and maternal hyperpyrexia.

Significance to Biomedical Research and Program of the Institute: As the result of applying higher standards of medical care to obstetrics during the past thirty years, maternal mortality has decreased tenfold. Over the same period, perinatal mortality has remained relatively unchanged even through similar high standards of medical care have been employed. In 1964 in the United States, from a total of about 3.7 million births a year, there were 28 perinatal deaths per 1,000 live births; the perinatal death rate in 1967 was 26.2 per 1,000 live births. This number of deaths is matched by a similar amount of perinatal morbidity which includes respiratory distress syndrome, mental retardation, congenital heart disease, and other malformations.

Since a large proportion of perinatal mortality is associated with the last week of gestation, it is pertinent to examine current methods of checking fetal condition during the course of labor. During the last decade, biochemical and biophysical techniques, separately and in combination, have been proposed as methods of fetal surveillance during labor and delivery. Since a sizable proportion of perinatal morbidity and mortality may be directly related to the stresses of labor and delivery, it is important that the relative merits of these fetal monitoring techniques for fetal and neonatal survival and well-being be determined, particularly in relation to specific high-risk obstetrical conditions.

The complementary use of biochemical and biophysical methods to study the fetus, during labor and delivery, and the infant through the period of extrauterine adaptation provides valuable information not previously available. Information obtained will aid in delineating the respective value

of biochemical and biophysical techniques for fetal surveillance in specific types of high-risk pregnancies.

Proposed Course: Subjects are selected from "high-risk" patients who have diagnoses including toxemia, chronic hypertension, Rh sensitization, abruptio placenta, maternal diabetes, premature rupture of the membranes, premature labor, prolonged pregnancy, para 6 or greater, elderly primagravida, breech presentation, previous cesarean section, and maternal hyperpyrexia. High-risk patients are viewed from the standpoint of the Goodwin high-risk score and the specific high-risk indication, e.g., toxemia, and compared with patients without complicating obstetrical conditions.

Basic biophysical (FHR, FECG, UC) and biochemical (pH, pO_2, pCO_2) data are collected from detailed study of the mother and fetus during labor and from the newborn through the first hour of life. Maternal and neonatal condition is assessed at 24 and 48 hours following delivery.

Electronic data processing facilities are used to handle data accruing from these investigations. Data are being subjected to descriptive and statistical analyses (correlations, Chi-squares, and multiple regression).

A detailed profile is to be developed on each group of high-risk indications so it will be possible to consider the group as a whole or to compare one indication with another.

As of March 31, 1972, 146 new patients have been monitored. All data (except FECG values) from 85 of these patients have been analyzed. Data from the remaining 61 patients are currently being examined. In addition, data from 35 pateints that had been studied previously have been analyzed. Delay in receipt of equipment necessary for FECG analysis has slowed down the FECG analysis. This work is now moving ahead; to date preliminary results have been obtained on 20 patients.

NICHD Annual Report
July 1, 1971 through June 30, 1972
Adult Development and Aging Branch

The congressional discussion that preceded the establishment of NICHD stated that the Institute would be responsible for biological, medical, and behavioral aspects of aging.

Biological aging processes cause the deterioration that gradually changes young adults into old ones. They produce much disability in themselves and contribute to the development of many diseases of the middle-aged and elderly. The study of these biological processes is a central feature of NICHD programs in aging.

In general, the Institute does not support research on individual diseases since these are covered by the disease-oriented Institutes of NIH. NICHD's concern with the diseases of the elderly is much broader, covering morbidity and mortality of all the individuals who make up the population. In other words it is concerned with diseases and their interrelationships as they impinge on human development.

In addition to studies of the processes of cognitive change with age, the Institute is responsible for studies that bear on the problems of psychological adjustment that humans face as they pass from one life stage to another. It is also responsible for studies in the social sciences relating to the problems that the aging of our society pose for the individuals who are aging and for the society as a whole. There is much in this area of responsibility that also falls within the mission of other agencies. NICHD is particularly concerned with the development of a central body of sociological theory and fact that will make possible a comprehensive view of the problems of the aging and the elderly and an appropriate approach to the solution of those problems.

GROWTH OF EXTRAMURAL RESEARCH ON AGING

The growth of research and training grant programs in terms of budgetary expenditures (in thousands of dollars) is tabulated below.

FY	Research Grants	Training Grants	Contracts	Total
64	$2,735	$301	$0	$3,036
65	2,625	567	141	3,333
66	3,210	1,450	59	4,719
67	3,234	2,089	102	5,425
68	3,502	2,197	213	5,912
69	3,485	2,286	325	6,096
70	3,226	2,314	485	6,025
71	3,801	2,232	240	6,273
72*	4,707	2,450	907	8,060

*Estimated

RESEARCH AND TRAINING PROGRAMS

This section will discuss various topics of importance in research and training and will list the research and training supported in FY 72 so that the structure of the program can be seen.

Society, Aging, and Health

The control of infectious and nutritional diseases in this century has been a major factor in the striking alterations that have taken place in the size and age structure of the American population. The United States now has a much larger fraction of older persons in its population than it did at the turn of the century, and much of their morbidity and mortality comes from diseases that had relatively small impact on their ancestors.

Continuing social and medical advances may be expected to continue to change the age structure of the population and the relative importance of different diseases. Some efforts to foresee what may be expected have been made, but the efforts have been small, and there has been little systematic planning on the medical, psychological, and societal consequences of improved prevention and treatment of the diseases that are now important.

Eliminating cancer would add about 2.3 years to the expectation of life at birth in the U.S. Elimination of cardiovascular-renal diseases would increase the current expectation by 10.9 years. These increases would, of course, tend to increase the fraction of older persons in the population. Equally and perhaps more important in changing the age-structure of the population may be the birthrate. Mathematical models will be necessary to quantitatively project the age-structure that will be produced by alterations in fertility, mortality, and migration. Without such models one can only say that probably a larger fraction of the population will be older, that a different pattern of diseases will emerge, and that intrinsic aging processes will become more important.

NICHD is planning a program that will provide a broad conceptual background for persons concerned with the implications of changing population structure for health and other social problems. Currently it is funding two contracts to review, analyze, and evaluate past and current research in specified areas and to make recommendations for future research in those areas. The areas concern (1) mathematical models for predicting changes in population size and structure as a result of alterations in fertility, migration, and mortality rates, (2) estimates of possible future values for fertility, migration, and mortality, and (3) predictions of the impact that changes in size and structure of the population will have on health, needed health research, public health measures, and health services.

Mental Function

One of the major problems of many old persons both from their own standpoint and from that of society is impaired mental function which may progress to the point of dementia and completely incapacitate those affected. Much remains to be learned in this area. Impairment in cognitive ability probably begins

in many persons at least by the middle years of life. In late life a significant number of persons are severely demented.

Whether these dementias are in part the end result of the cognitive decline that begins relatively early in life or whether they for the most part are due to other processes is unknown. It has been thought for many years that a large fraction of the senile dementias are due to atherosclerotic vascular disease. However, many neuropathologists are now beginning to believe that much of what was attributed to vascular disease is due to primary neuronal disease. NICHD supported a conference dealing with such primary neuronal disease and aging at the Winter Conference on Brain Research held at Vail, Colorado in 1972.

NICHD supports a moderate amount of research on the cognitive defect of age and is supporting studies of the effect of the inhalation of 100 percent oxygen under increased pressure on the mental function of severely demented elderly persons. There is evidence that this may prove of value, but many more studies need to be done before this therapy can be considered anything other than experimental.

Aging in Women

Last year NICHD supported a conference to lay the foundations for a program to study the causes, manifestations, and treatment of the results of the menopause. At the conference the hormonal changes associated with the menopause, their possible relations to atherosclerosis, osteoporosis, and other diseases, and the possible advantages and disadvantages of replacement therapy were discussed. This year a retrospective study of the effects of replacement therapy, and a study of the hormonal alterations in the post-menopausal period were started.

Aging and Immunology

There is considerable evidence that immunological competence declines with age and that the decrements that occur are important in some of the major manifestations of old age. The level of activity of both the cellular and humoral immune systems peaks during adolescence and thereafter declines. These decreases in immunological competence are very important in the diminished resistance of the elderly to infections and very possibly contribute to the high incidence of cancer in the elderly. An increased understanding of the processes involved might well make possible procedures to strengthen immunological competence in old age and thus to improve the health of the elderly. NICHD began the support of several studies in this area in FY 72.

Cellular Aging

Changes at a cellular level are probably responsible for many of the aging changes that occur at the level of the whole person and are so easily apparent to layman and to physician. Thus the Institute has emphasized research on the cellular processes of aging.

Since the limited generation potential of certain mammalian cells appeared to be an important lead, emphasis was placed on understanding this phenomenon. Programmatic efforts in this area began in 1968.

At one time fibroblasts were thought to be potentially immortal in tissue culture, but this has proved to be not true of normal human fibroblasts. It is now known that they undergo only about fifty doublings in vitro before dying. It is also known from transplantation studies that at least one cell type, mouse mammary epithelium, will not continue to divide indefinitely when transferred serially from young mouse to young mouse.

NICHD has held a number of meetings of investigators in this field to develop an appropriate program dealing with this phenomenon. It supports the production of cells suitable for studying it. In the summer of 1972 it supported a course on the use of tissue culture in studies of aging. This course was given at the University of Vermont. NICHD now supports a moderately large program of research in this area by the research grant mechanism.

Animals for Aging Research

A major limiting factor in the expansion of experimental work on aging has been the difficulties involved in getting old animals. This factor has created serious problems for investigators with a long-term commitment to aging research and has prevented investigators without great interest in aging from introducing age as a variable into their studies.

Most experimental work on aging has been carried out on rats and mice for what appear to be good reasons. They are mammals and therefore results obtained with them would appear to stand a better chance of being relevant to human aging than results obtained with non-mammalian species. Their relatively short lifespans are important both in the conduct of experimental work and in the cost of raising them into old age. Their small size makes it relatively inexpensive to raise them. They have been widely used in other types of biological studies and therefore are well-characterized in many ways.

However, the difficulties in raising rodents into old age are much greater than might be anticipated. The major problem has been acute and chronic infectious diseases. These impair health and shorten life. Their presence in colonies in the past led to animals being considered old when they were in middle age, and to their being studied for age changes when their functional status was deranged by disease.

Techniques are now available to raise rodents free of parasites and either free of bacteria or with a defined bacterial flora. However, the methodology requires a large investment of time and facilities. It is therefore not practical for most investigators to breed their own animals and raise them to old age. Therefore, NICHD has exerted much effort to make it possible for investigators to obtain well-characterized, healthy, old rats and mice from commercial breeders.

NICHD now has contracts with a commercial breeder to make this possible. It supports the characterization of inbred Fischer-344 rats, the inbred C57BL6 mouse, the inbred BALB/c mouse, an F1 hybrid mouse based on the two inbred mouse lines, and the outbred ICR mouse. The animals are free of bacterial and mycoplasmal disease. Animals of various ages are periodically selected

randomly and autopsied. Both gross and histological examinations are made. Appropriate bacteriological, serological examinations for viral infections, and parasitological examinations are carried out. A number of these animals will be kept through their lifespans so that investigators using these strains will have models whose natural histories and life tables are well characterized.

Training Programs

In FY 72 NICHD supported 21 grants for training in aging research. Training was approved for a total of 146 predoctoral and 56 postdoctoral trainees. These were distributed as shown in the table below.

Number of Trainees

	Biological Sciences	Behavioral Sciences	Total
Predoctoral	26	120	Pre= 146
Postdoctoral	34	22	Post= 56
	60	142	202

NICHD has supported a summer course on the biology of aging since 1969. The faculty each year is composed of scientists from a number of universities. The course has been a very satisfactory mechanism for bringing together scientists with a long-standing interest in aging and scientists not previously involved in aging but with outstanding relevant competence.

RESEARCH GRANTS* IN FY 72

Research on Behavioral Aspects of Aging

Grants in this area were:	Direct Costs
Lowenthal: Adult Development--Continuities and Discontinuities	$132,152
Jacobs: Hyperoxygenation Effect on Recent Memory in the Aged	55,309
Schaie: Cognitive Behavior in Maturity and Old Age	16,550
Fozard: Mental Performance and Aging	51,957
Eichorn: Intergenerational Studies of Development and Aging	95,900
Eisdorfer: An Integrated Investigation of Aging and the Aged	468,821
Ostfeld: Relocation in Old Age- Health and Psychosocial Impact	72,496
Davis: Aging in Monkeys	5,500
Botwinick: Psychophysiological Aspects of Aging	29,715
Crockett: Personal Constructs, Aging, and Impression Formation	28,464
	$956,864

*Only Direct Costs are given. Several grants may be funded in FY 72 after the submission of this report.

Research on Cellular Aging

The grants in this area were:

Adelman: Biochemical Regulation of Aging Processes	$91,000
de Duve: Mechanisms of Cellular Aging and Degeneration	153,969
Gottlieb: Enzyme Activities in the Development of Fungi	29,039
Packer: Aging Mechanisms of *in vitro* Human Cell Systems	201,635
Lansing: Experimental Cytology of Aging Cells and Tissues	50,552
Wright: Computer Analysis of Differentiation	33,560
Adelman: Effects of Senescence in Enzyme Regulation	14,906
Cristofalo: Carbohydrate Metabolism and Aging *in vitro*	39,010
Saunders: Morphogenetic Cellular Death	45,000
Brandes: Function of Lysosomes in Relation to Aging	73,300
Comings: Aging and DNA - *in vitro* Studies	27,824
Hayflick: Programmed Senescence in Cultured Human Cells	48,227
Kelvecz: Temporal Order and Aging in Cultured Somatic Cells	21,050
Miller: Cell Energetics and Aging	22,800
Widnell: Membrane Function in Aging and Development	24,125
Cristofalo: Cell Senescence and the Control of Cell Proliferation	375,701
Masaro: Role of Biological Membranes in Aging Processes	143,172
Daniel: Studies on the Aging of Mammary Epithelium	22,534
	$1,417,404

Research on Aging of Extracellular Matrix

Tonna: Skeletal Cell and Matrical Changes during Aging	$59,107
Hruza: Metabolism of Lipids and Calcium during Aging	51,222
Robbins: An Interdisciplinary Program of Research in Aging	260,774
Meyer: Mucopolysaccharides of Connective Tissues	127,903
Federman: Changes in Cell Type and Structure in Developing Bone	35,079
Klein: Collagen Biosynthesis during Bone Remodeling and Aging	50,000
	$584,085

Miscellaneous Biological Research on Aging

Rockstein: Aging in *Musca Domestica*	$26,143
Bierman: Gerontology Research Program - Metabolism and Aging	133,900
Hembrough: Gerontological Studies in Animals	40,010
Niehaus: Lipid Peroxidation - Mechanism and Role in Aging	18,100
Kreilick: Hindered Phenols as Radical Scavengers and Spin Labels	23,060
Lang: Biological Aging Program - Louisville	54,770
Romanoff: Effects of Aging on Steroid Metabolism in Man	35,234
Paffenbarger: Factors in Youth Predisposing to Chronic Diseases	52,187
Dill: Age-Related Responses to Environmental Stress	12,000
Sanadi: Metabolic and Molecular Changes in Aging	116,338
Hayashida: Studies with Growth Hormones and Prolactin	53,605
Willis: Developmental Analysis	19,953
Srakotos: Developmental Changes in Dipteran Lipoproteins	29,069
Sinex: Aging in Connective Tissue, Brain, and the Auditory System	209,329
Rothstein: Studies of Aging in a Nematode Model System	163,642
	$987,340

TRAINING

The Training Grants were:

Schaie	Life Span Developmental Psychology	$65,016
Botwinick	Psychology-Geriatrics	127,916
Timiras	Developmental Physiology and Aging	57,899
Neugarten	Gerontology	212,025
Rockstein	Gerontology	77,160
Meyer	Developmental Psychology	42,406
Birren	Multidisciplinary Approach to Gerontology	246,998
Eisdorfer	Behavior and Physiology in Aging and Human Development	180,000
Peterson	Social Gerontology	106,428
Taylor	Social Gerontology	53,530
Curtis	Gerontology	68,740
Sinex	Biochemistry of Aging	45,400
Horvath	Physiology of Exercise and Stress	70,640
Lowenthal	Adult Development and Aging	130,660
Hamilton	Aging and the Endocrine System	37,061
Sanadi	Biochemical and Biophysical Basis of Aging	89,609
Makinodan	Aging	101,279
Eisdorfer	Gerontology Society Course on Behavioral Aspects of Aging	42,813
Kohn	Aging of Mammalian Tissues	43,220
Huang	Age-Dependent Nucleo-Cytoplasmic Interaction	57,114
Rosenberg	Social Gerontology	22,475
	Total	$1,878,389

The Fellowships were:

Walton	Cyclic AMP in Development and Aging	
Moore	Age and Social Change - Ethnic Identity	
Stein	Senescence and the Accumulation of Errors in Proteins	
Huzar	Regulation of Collagen Biosythesis in Development	
Smith	A Biochemical Study of Senescence in Fish	
Dudl	Effect of Aging on Carbohydrate Metabolism	
Terman	Study Basic to Aging of Immuno Surveillance and Cancer	
Bradley	Mechanisms of Aging as Studied by Cell Fusion	
Kalish	Social Gerontology of Religion and the Clergy	
	Total	$97,523

The Research Career Development Awards were:

Gutman	Comparative Study of Ego Processes in Later Life	
Lang	Biochemical Changes during Growth and Aging	
Holloszy	Exercise Induced Biochemical and Anatomic Adaptations	
Lieberman	Adaptation and Survival under Stress in the Aged	
Kohn	Aging Mechanisms of Connective Tissue	
Schaiberger	Biochemical Changes in Cells with Aging	
Binstock	American Political System and Health of the Aging	
	Total	$181,979

CONTRACTS IN FY 72

(University of Texas) Estrogen in the Postmenopausal Woman	$125,750
Terry (Yeshiva University)	
Workshop on Aging and Senility in the Nervous System	4,932
Jackson (Duke University	
Research Conference on Minority Group Aged in the South	5,000
McFarland (University of Chicago)	
Mathematical Models for an Aging Population	35,985
Morris (Brandeis University)	
Conference on Regional Usage of Gerontological Centers for Research	6,000
Birren (University of Southern California)	
Role of Institutions of Higher Learning in Problems of Aging	10,000
Meyers (Duke University)	
An Interdisciplinary Workshop on Demographic Problems of Aging	9,640
Bell (Veterans Administration, Boston)	
Longitudinal Studies	6,601
Smith (East Carolina School of Medicine, Greenville, N.C.)	
Accelerated Immunological Aging: A Possible Model for Research in Relation to Rejuvenation by Fetal Liver	38,874
Hayflick (Stanford University)	
Production, Characterization, and Distribution of Human Diploid Cell Strains	105,624
Absher (University of Vermont)	
Quantitative Studies of Aging Human Diploid Fibroblasts in vitro	72,419
Daniel (University of California, Santa Cruz)	
Fourth Annual Course on the Biology of Aging	50,375
Teague (University of Florida, Gainesville)	
Analysis of Aging Associated Involution of Cellular Immunity in Mice	100,787
Stinebring (University of Vermont)	
Cell Culture Procedures for Aging Research	54,567
Horvitz (Research Triangle, North Carolina)	
Review, Analysis and Evaluation of Population Models	30,000
Meyers (Duke University)	
Review, Analysis and Evaluation of Population Models	50,000
(American Psychological Association)	
Conference	4,932
Eisdorfer (Gerontological Society)	
Travel for IAG Conference	50,000
Foster (Charles River Laboratories)	
Contract to Breed, Rear, and Maintain a Colony of Inbred Aging Laboratory Rats for Aging Research	40,700
Foster (Charles River Laboratories)	
Production Colony of Aging Rats in an Isolator Environment	10,942
(University of California, Irvine)	
A Retrospective Study of Postmenopausal Women With and Without Estrogen Replacement Therapy	52,368
Foster (Charles River Laboratories)	
Development of a Production Colony of Two Inbred an F1 Hybrid, and an Outbred Laboratory Mouse for Aging Research	No FY 71 Money
Total	$865,496

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ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: 72-C-399

CONTRACT TITLE: Workshop on Aging and Senility in the Nervous System

CONTRACTOR: Yeshiva University, Bronx, New York
(Contract Officer: Dr. Robert D. Terry)

MONEY ALLOCATED: $4,932

OBJECTIVES: Although there has been increased interest in research on aging in the nervous system, this research has tended to be compartmentalized by traditional disciplines. Thus neuropathologists and neuroanatomists have had little contact with neurochemists and immunologists. In order to bridge these gaps this workshop was convened to meet concurrently with the Winter Conference on Brain Research. This meeting was selected because it was then possible to hold a workshop session as part of the Brain Research meeting which served to introduce other neuroscientists to the problem of aging in the nervous system.

SIGNIFICANCE FOR AGING RESEARCH: The workshop will give rise to further contacts and to increased research in aging in the nervous system.

PROPOSED COURSE: This contract was for one year.

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ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: 72-3-016

CONTRACT TITLE: Accelerated Immunological Aging: A Possible Model for Research in Relation to Rejuvenation by Fetal Liver

CONTRACTOR: East Carolina School of Medicine, Greenville, North Carolina (Contract Officer: Dr. A. Mason Smith, Department of Microbiology)

MONEY ALLOCATED: $38,874 (2 years)

OBJECTIVES: The contractor will exert its best efforts to:

1) Determine anti-sheep RBC responding capacities and levels of IgM IgG, and IgA in the blood of several strains of mice at 0.5, 1, 2, 4, 8, and 12 months of age. Conduct a comparative study on the following strains of mice: SJL/J, BALB/C, mice congenic with BALB/C, and C57BL and their hybrids;

2) Perform autopsies on lymphoidal tissues for pathological diagnosis;

3) Attempt to impair the immune system by injecting at various ages heterologous anti-heavy chain sera and, if successful, determine if the rate of recovery is age-related;

4) Attempt to rejuvenate the immune system of aged mice by injecting fetal liver and thymus from syngeneic and congenic donors. The fate of the donor cells will be assessed; and

5) Determine the frequency of incidence of reticulum cell sarcoma, the levels of immunoglobulin classes, and the anti-sheep RBC responding capacity in mice injected with fetal liver and thymus.

SIGNIFICANCE FOR AGING RESEARCH: There is evidence that immunologic competence declines with age. Since immunologic diseases -- neoplastic, autoimmune, and infectious -- show increased incidence with age, it is important to understand the genesis of this age-related decrease in immune competence. The inability of the organism to control such diseases is likely associated with a functional change in immunologically reactive cells as well as with a decrease in ability to mount a rapid and intense immune response as would be required for infectious diseases. Studies should be encouraged to attempt to determine the mechanisms by which immunocompetence develops, the variables that modify the rates of its development, the significance of loss of competence, and possible methods of therapeutic intervention.

PROPOSED COURSE: This contract should be maintained active at least two years.

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ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: 69-2091

CONTRACT TITLE: Production, Characterization, and Distribution of Human Diploid Cell Strains

CONTRACTOR: Stanford University, Stanford, California
(Contract Officer: Dr. Leonard Hayflick)

MONEY ALLOCATED: (9/30/71 - 11/30/72): $105,624

OBJECTIVES: This resource is designed to encourage the development of high quality research in the field of cell aging by providing well-characterized cultured human cells to, as well as accelerated information exchange among, the community of investigators studying the phenomenon of aging in the human diploid cell. Starter cultures may be obtained on a noncompetitive basis whereas more extensive needs for material are subject to merit review and competitive allocation of the resource.

SIGNIFICANCE FOR AGING RESEARCH: This contract has been singularly effective in stimulating the development of a specialized field of research addressing cell aging phenomena in diploid human cells in culture. The contract has passed from the stage of being highly speculative, and of introducing new investigators to the field, to one where the major capacities of the resource are being consumed by investigators who are conducting long-term research on this cell strain and who depend upon this contract as a source of characterized and standardized cell materials. The state of this service will be under close scrutiny in the coming months to determine the possible desirability of policy changes and/or location changes. A certain amount of expansion of cell production capability appears necessary. To achieve this, Dr. Hayflick's laboratory may require considerable capital outlay. Alternative approaches to increase cell production include more extensive use of commercial suppliers such as HEM, Inc., or change to the facilities of an investigator such as Dr. Cristofalo. This latter alternative is particularly desirable in view of Dr. Cristofalo's 1) competence, and 2) proximity to many of the heavy users of the resource. The annual workshop held on this contract has been highly effective. The next is planned for Spring 1972 at Asilomar Conference Grounds in California.

Policy and substance of this contract have been subject to the review and deliberations of an ad hoc advisory panel which meets one or two times a year in addition to the formal contract review session. The three members of the panel are: Dr. Paul Kruse, Samuel Roberts Nobel Foundation, Route 1, Ardmore, Oklahoma 73401; Dr. George Martin, Department of Pathology, University of Washington, Seattle, Washington 98105, and Dr. David Axelrod, Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201.

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ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: NIH-NICHD-72-2755

CONTRACT TITLE: Quantitative Studies of Aging Human Diploid Fibroblasts *in vitro*

CONTRACTOR: University of Vermont, Burlington, Vermont
(Project Officer: Dr. Marlene Absher, Department of Medical Microbiology)

MONEY ALLOCATED: $72,419

OBJECTIVES: To address details of cell population dynamics of the human diploid fibroblast strain WI-38 or equivalent with particular attention to:

1. Clonal and stemline division patterns at representative passage levels with particular attention to sequences terminating in non-dividing cells.

2. Fate of non-dividing cells.

3. How cell division patterns contribute to observed population dynamics at Phases I, II, and III.

SIGNIFICANCE FOR AGING RESEARCH: The goal of this program is to understand the mechanisms of human cellular aging and, ultimately, the consequences of these processes at the tissue, organ and organism levels. A number of biological models are particularly attractive for studies of cellular aging and, of these, *in vitro* studies of human diploid fibroblast-like cells (HDF) have become a focus of interest in the scientific community concerned with program support of program project grants, project grants, and two service contracts providing an HDF resource, communicative resource, and training in cell culture procedures. The rapid expansion of studies addressing the aging phenomenon of the HDF has resulted in an unequal distribution of research effort with a consequence of gaps in knowledge fundamental to the descriptive characterization of the systems: notably cell population dynamics. These descriptive data are apparently obtainable with existing techniques, and are of probably major consequence to current and projected research programs.

This contract is to support studies seeking these data.

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ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: Unassigned

CONTRACT TITLE: 4th Annual Course on the Biology of Aging

CONTRACTOR: The University of California, Santa Cruz
(Contract Officer: Dr. Charles W. Daniel)

MONEY ALLOCATED: $50,375

OBJECTIVES: This contract provides for a course in the biology of aging. The course is aimed at stimulating interest in the field of aging. The course will bring together a select group of faculty, whose contribution to the field of aging or to science in general is well recognized, and an equally select group of 25 advanced students, who have yet to make a substantial contribution to aging research, but who, given the proper stimulus, may well do so.

The format is designed to stimulate mutual exchange of information and ideas. An intensive lecture schedule with not unlimited but adequate time for questions and comments and panel discussions in the evenings should allow for a significant amount of interchange and for an intensive, open-ended dialogue. Ample opportunity for individual contact will be possible since all faculty and students will be housed and have meals together.

SIGNIFICANCE FOR AGING RESEARCH: The many significant questions underlying the mechanisms of aging can now be formulated in such a way that there is some likelihood that some may be answered in the future. The biology of aging is an emerging field with a conspicuous lack of basic information and would benefit from the contributions of young scientists. This course provides the opportunity for scientists to gather for two weeks of intense discussion on the aging problem.

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ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: 72-3-008

CONTRACT TITLE: Analysis of Aging Associated Involution of Cellular Immunity in Mice

CONTRACTOR: University of Florida, College of Medicine, Gainesville, Florida
(Contract Officer: Dr. Perry O. Teague, Department of Pathology)

MONEY ALLOCATED: $100,787 (2 years)

OBJECTIVES: The contractor will exert its best efforts to:

1) Determine whether or not thymic epithelial cells and/or uncommitted bone marrow lymphocyte stem cells decline in ability to function in normal cellular immune responses with aging;

2) Determine if an active thymus is required for retention of "normal thymus dependent cellular immune functions" during aging of thymectomized and of thymectomized and sublethally irradiated mice;

3) Determine whether thymic epithelial cells or bone marrow stem cells from young and old syngeneic donors will reconstitute thymectomized and lethally irradiated mice of three strains -- A/J, C57Bl/6J, and DBA/1J, and

4) Determine if spleen cells from each mouse strain respond to stimulation in vitro to PHA, SEB, and allogeneic mitomycin-C-treated spleen target cells.

SIGNIFICANCE FOR AGING RESEARCH: There is evidence that immunologic competence declines with age. Since immunologic diseases -- neoplastic, autoimmune, and infectious -- show increased incidence with age, it is important to understand the genesis of this age-related decrease in immune competence. The inability of the organism to control such diseases is likely associated with a functional change in immunologically reactive cells as well as with a decrease in ability to mount a rapid and intense immune response as would be required for infectious diseases. Studies should be encouraged to attempt to determine the mechanisms by which immunocompetence develops, the variables that modify the rates of its development, the significance of loss of competence, and possible methods of therapeutic intervention.

PROPOSED COURSE: This contract should be maintained active at least two years.

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ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: Unassigned

CONTRACT TITLE: Cell Culture Procedures for Aging Research

CONTRACTOR: University of Vermont, Department of Medical Microbiology
(Project Officer: Dr. Warren Stinebring)

MONEY ALLOCATED: $54,567

OBJECTIVES: This course is designed to enhance NICHD program activities in the field of cellular aging by providing an optimum training experience in cell culture and cellular aging. The course is to be 3 weeks in duration (10-30 September, 1972) and offers advanced lecture presentations and intensive laboratory instruction.

REMARKS: 24 participants and several auditors will be selected on a competitive basis. The course is the second in a series co-sponsored by the NICHD and the Tissue Culture Association (TCA). The 1972 course encompasses changes recognized as desirable from the experience of the previous course (held at the W.A.J. Cell Science Center, Lake Placid, New York) and is responsive to current needs in the field. A planning meeting for the 1973 course was held jointly by the NICHD and the TCA April 21, 1972 at Wistar Institute, Philadelphia. This third course in the series will be more advanced in subject matter and of shorter duration (2 weeks).

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: 72-C-199

CONTRACT TITLE: Research Conference on Minority Group Aged in the South

CONTRACTOR: Department of Psychiatry, Duke University, Durham, North Carolina
(Contract Officer: Dr. Jacquelyne Jackson)

MONEY ALLOCATED: $5,000

OBJECTIVES: The specific objectives of the conference were (a) the presentation of the current status of research, training and services relative to black aged; (b) concentration upon present gaps; and (c) initiating action to help reduce gaps through the development of graduate training programs in social gerontology.

Four major forms of presentation and discussion were utilized: formal seminar presentations and discussions, invited addresses, small group workshops and informal discussion. The conference lasted for two days and a report of the meeting is being prepared.

SIGNIFICANCE FOR AGING RESEARCH: This contract will stimulate more research interest in aging minorities.

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: Unassigned.

CONTRACT TITLE: Mathematical Models for an Aging Population

CONTRACTOR: University of Chicago, Chicago, Illinois
(Contract Officer: Dr. David McFarland)

MONEY ALLOCATED: $35,985

OBJECTIVES: Planning for future health research and services for an aging population depends heavily upon projections of the future size and age structure of that population. This contract supports the review, analysis and evaluation of existing mathematical population models, especially in regard to the feasibility of their use in connection with projection and planning of health research and services for the middle-aged and elderly. The review will include a number of currently unpublished works known to be in progress from the viewpoint of ascertaining the manner in which each model incorporates those members of the population in the upper age groups and the manner in which each model incorporates mortality from various causes.

Recommendations for new research to be conducted will be made.

SIGNIFICANCE FOR AGING RESEARCH: The contract should provide information on the current status of research on mathematical models for predicting population size and structure and an evaluation of the models in terms of their applicability for research relating to the elderly.

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: Unassigned

CONTRACT TITLE: Conference on Regional Usage of Gerontological Centers for Research

CONTRACTOR: Brandeis University, Waltham, Massachusetts
(Contract Officer: Dr. Robert Morris)

MONEY ALLOCATED: $6,000

OBJECTIVES: This contract supports a conference which considers the extent to which regionalization of gerontological research institutes and centers can be carried forward. The regionalized approach is one in which several existing centers undertake the dual task of continuing their activities and functioning as the nucleus for strengthening the gerontological developments in the other universities in their regions.

The purpose of the conference is to (1) consider how activities of major centers in gerontology can be better coordinated, especially their research activities and (2) the potential for expanding the regional contribution which existing centers can make to other gerontology oriented centers in their regions.

SIGNIFICANCE FOR AGING RESEARCH: This contract should provide information on the best way to take full advantage of the developing centers for aging research.

PROPOSED COURSE: This contract is for one year.

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ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: Unassigned

CONTRACT TITLE: Role of Institutions of Higher Learning in Problems of Aging

CONTRACTOR: Ethyl Percy Andrus Gerontology Center, University of Southern California, Los Angeles, California
(Contract Officer: Dr. James E. Birren)

MONEY ALLOCATED: $10,000

OBJECTIVES: Representatives from institutions of higher learning will be brought together to discuss prospects and mechanisms for training in aging. The conference will involve administrative persons, faculty members in various disciplines, graduate students, representatives of agencies employing personnel in aging, and representatives of specialized organizations that deal with the needs of an aging population who might be able to define needs and goals.

The proceedings of the conference will be published. It will include the invited papers from major speakers and the ensuant discussions.

SIGNIFICANCE FOR AGING RESEARCH: The conference should provide impetus to greater cohesiveness between training, research and services in behalf of the needs of the elderly.

PROPOSED COURSE: This contract will be awarded for only one year.

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ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: Unassigned

CONTRACT TITLE: Travel - International Congress of Gerontology

CONTRACTOR: American Gerontological Society
Washington, D.C.
(Contract Officer: Mr. Edwin Kaskowitz)

MONEY ALLOCATED: $50,000

OBJECTIVES: This contract provides for partial support of travel of 75 American scientists to the 9th International Congress of Gerontology in Kiev, USSR, July 1-7, 1972. The International Association has held a scientific congress each third year since 1950 and each has resulted in the publication of Proceedings which have had world wide distribution.

Planning for the meeting has been on an international basis with a broad multidisciplinary representation. It is important to have a good representation of American scientists at the meeting.

SIGNIFICANCE FOR AGING RESEARCH: The impetus of this international congress will provide for increased international communications, sharing and at times collaborating on research and program developments in aging on a cross-national and cross-cultural basis.

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ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: Unassigned

CONTRACT TITLE: An Interdisciplinary Workshop on Demographic Aspects of Aging

CONTRACTOR: Duke University, Durham, North Carolina
(Contract Officer: Dr. George Myers)

MONEY ALLOCATED: $9,640

OBJECTIVES: This workshop will bring together a selected group of researchers in the fields of aging and demography. The purposes of this workshop are to:

1. Establish a dialogue between researchers in aging and demography.

2. Review current knowledge regarding the aging process and demographic approaches to its study.

3. Determine major problem areas in need of research and recommend priorities for research.

4. Prepare a report.

SIGNIFICANCE FOR AGING RESEARCH: The workshop will focus research on important multidisciplinary topics related to aging and demography.

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: (Reimbursable Agreement)

CONTRACT TITLE: Longitudinal Studies

CONTRACTOR: Veterans Administration, Boston, Massachusetts
(Contract Officer: Dr. Benjamin Bell)

MONEY ALLOCATED: $6,601

OBJECTIVES: This contract supports several meetings involving investigators of the longitudinal studies of aging. The purpose of the meetings is to discuss topics of common concern to investigators in longitudinal studies, such as procedures and problems in collecting and utilizing longitudinal data. Each of the meetings involves a small number of working investigators who share a common interest and responsibility.

SIGNIFICANCE FOR AGING RESEARCH: Comparisons of procedures and data would do much to clarify the important differences and similarities among the various study populations. The conferences would serve as a coordinating mechanism for the longitudinal studies on aging.

PROPOSED COURSE: This contract should be maintained on a continued yearly basis.

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Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: 70-2097

Contract Title: Development of a Production Colony of Three Genotypes and a Non-Inbred Strain of Laboratory Mouse for Aging Research

Contractor: Charles River Breeding Laboratories, Wilmington, Massachusetts

Money Allocated: $10,486 Continued into FY 1973

Objectives: 1. Provide characterized genetically defined strains of laboratory mice reared in a defined environment for research in aging.

2. Develop a ready commercial source of aging mice of three basic genotypes and an outbred strain to meet the demands for aging laboratory mice.

3. Minimize lag time for the development of studies in aging requiring aged genetically defined laboratory mice from a controlled environment.

4. Provide the minimum number of strains of mice necessary for cross comparison and extrapolation of experimental results to a broader natural population.

5. Develop a colony of laboratory mouse strains in which pathological processes, degenerative change, morbidity and mortality to age 24 months are largely known and predictable.

Significance for Aging Research: A lack of aged genetically and biologically defined animals reared in a controlled environment has long hampered the development of aging research, particularly in the field of immunology. With increasing frequency studies in aging research require animals of known genetic background, biological characterization and environmental status. To meet this need for strains of genetic specificity, diversity and generalizability a colony of aging mice of the inbred strains C57/BL6, BALB/c, the inbred F_1 hybrid of the two inbred strains and the outbred strain ICR was established in a barrier enclosure (SPF) at Charles River Breeding Laboratories. Profile data will be acquired on the colony and strains of animals by periodic sacrifice and necropsy.

The major significance of this contract is the development of a readily available resource of aging, genetically defined and characterized strains of laboratory mice reared in a controlled environment. The standing colony of aging mice of the three genotypes and non-inbred strain proposed under this research contract will provide investigators in aging with basic genetically controlled model systems previously unavailable to most investigators in aging. This should moderate one of the primary constraining influences on

the development of aging research in animals by making available: 1) basic genetic model systems of the aging laboratory mouse for studies in aging requiring specific, diverse or general genetic control, 2) for study, one or several comparative animal model systems within a species, 3) an animal of known biological characterization and environmental status. In addition, once the colony is established the lag time from proposal to execution of the study will be substantially reduced.

Proposed Course: Contract will continue for a minimum of one year with the contract becoming increasingly self-sustaining in subsequent years.

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Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: 72-2739

Contract Title: Contract to Breed, Rear and Maintain a Colony of Inbred Aging Laboratory Rats for Aging Research (Modified)

Contractor: Charles River Breeding Laboratories, Wilmington, Massachusetts

Money Allocated: $40,700

Objectives: 1. Meet current and projected demands for senescent laboratory rats reared on a defined diet in a specific pathogen-free environment.

2. Establish a standing commercial resource of senescent rats on which investigators can immediately draw for aged laboratory rats.

3. Develop baseline physiological and pathological characterization of the Fischer 344 rat over their full lifespan.

4. Establish survival curves for laboratory rats reared pathogen-free behind a defined barrier system.

5. Increase the numbers and ages of animals to be made available for studies in aging.

Significance for Aging Research: A major constraint influencing the development of aging research has been the almost total absence of an aged animal resource sufficiently characterized to meet the unique needs of aging research. The development of a colony of aging laboratory rats under this contract will significantly enhance the quality and quantity of aging research by providing aged animals that are reared in a defined environment on a standardized diet, free of pathogenic organisms, and characterized with regard to age-specific causes of death.

Basic to the development of studies in aging research in animals is a characterization of expected physiological and pathological changes that occur over the animals' full lifespan as well as life tables that accurately reflect survival at specific ages. A primary aim of this contract is to acquire this data and make it available to investigators in aging. With this information, a reasonable comparative assessment can be made as to whether the animals, strain or stock is suitable for studies in aging. Also, within reasonable limits, numbers of animals needed for statistical significance of studies can be readily established, thus minimizing the likelihood of supporting excessive numbers of animals or too few animals for statistical significance of the study.

Currently many investigators in aging cannot acquire aged animals short of rearing the animals themselves, nor are they able to maintain aging rats under the laboratory conditions necessary to allow the animals to survive long enough to observe truly senescent change with age. Also, competent young investigators more often than not cannot support aging colonies of rats until they successfully compete for research support. Without this resource many imaginative young investigators will continue to be excluded from research in aging simply because they are unable to identify an aged animal resource which they could use in the studies they propose in aging.

Proposed Course: Contract is to be continued for a minimum of two years with the contract becoming increasingly self-sustaining, and until age-specific causes of death and life tables for the colony and strain can be established.

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Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: Unassigned

Contract Title: A Retrospective Study of Postmenopausal Women with and Without Estrogen Replacement Therapy

Contractor: University of California at Irvine, Irvine, California

Money Allocated: $52,368

Objectives: The aim of the proposed epidemiologic study is to determine whether and to what extent estrogen usage tends to increase the risk of stroke in a population of postmenopausal women living in a retirement community. The contract provides for the support of a retrospective study of the incidence of cerebrovascular disease, and other factors which predispose to cerebrovascular disease in a specific and uniquely discrete population of 7000 postmenopausal women. Cases of cerebrovascular disease occurring in postmenopausal women will be identified and described (morbidity and mortality). The population of identified stroke cases will be compared with an appropriate control group to determine if the case group is significantly different from that of the controls. Also, to determine the role of likely risk factors, proximity, dose and duration of drug usage in affecting severity, or type of stroke.

Significance to Aging Research: Until recently, the long-term effects of estrogens in postmenopausal women have been a matter of conjecture or largely ignored. The proposed study will provide current data on comparative risk of cerebrovascular disease in postmenopausal women taking estrogens and postmenopausal women who are not using them, but are otherwise at comparable risk from other causes.

The significance of this project lies in the fact that a substantial percentage of the women in the population are taking estrogen-like medications and the initial goal of the study can be completed within a two year period. At present the value of estrogen-like medication as therapy for postmenopausal symptoms, for the prevention of vascular disease, and for the arrest of osteoporosis remains unproven. There is serious concern that such medication in commonly used dosage is a significant health hazard with specific reference to the three most common causes of death--heart disease, cancer and stroke. There are no data available on the questions posed by this proposal. The population selected is uniquely constructed to permit this type of investigation. Information of this type is extremely important, since it may serve to guide the medical care of the many millions of women over the age of 50 in the United States.

Proposed Course: The study is planned for a minimum of two years. Continuation is dependent on results from the first two years' study.

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Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: Unassigned

Contract Title: Origin and Action of Estrogen in the Postmenopausal Woman

Contractor: The University of Texas Southwestern Medical School, Dallas, Texas

Money Allocated: $125,750

Objectives: The long-range goal of this project is to determine qualitatively and quantitatively estrogen production in postmenopausal women and the mode of interaction of estrogens and their target tissues. Earlier studies have shown, in oophorectomized postmenopausal women, that the principal estrogen produced is estrone and that for the most part, this arises from the extragonadal, extra-adrenal aromatization of androstenedione. In normal postmenopausal women most of the androstenedione is secreted by the adrenal glands but in women with ovarian stromal hyperplasia it can be secreted by the ovary as well. Also, changes in estrone have been shown to be associated with a variety of factors including obesity, age, diabetes, hypertension, liver function and some ovarian neoplasms. In view of the acquisition of this earlier data, the primary objectives of the contract are aimed at:

1. Establishing the relative contributions of ovaries and adrenals to plasma androstenedione and the extent of conversion of androstenedione to estrone and estrone sulfate prior to and following oophorectomy.

2. Determining the chemical nature of the hormone product following aromatization of androstenedione.

3. Delineating the effect of obesity, aging, hypertension, diabetes, liver function and some ovarian neoplasms on the conversion of androstenedione to estrone and estrone sulfate.

4. Determining the capacity of a variety of non-endocrine tissues to achieve the conversion of androstenedione to estrone by <u>in vitro</u> incubation studies.

5. Evaluate the relative efficacy of substances potentially active as aromatase inhibitors in an <u>in vitro</u> system utilizing a placental aromatizing enzyme system to inhibit excessive conversion of androstenedione to estrone, that occurs in some postmenopausal women.

6. Examining the interaction of estrone and estradiol in target tissues such as human endometrium and immature rat uterus.

With the attainment of these contract objectives, the origin and action of estrogen in postmenopausal women can be clearly defined as well as quality

and quantity of estrogen production.

Significance for Aging Research: The results of this study should contribute to understanding of the physiology and pathophysiology of estrogen production after the menopause as well as its medical management. Neither the quantitative or qualitative characteristics of estrogen production in the menopause have been fully elucidated. It is clearly evident that the data on levels of endogenous estrogen production, the chemical nature of estrogens produced and their biological activity as well as factors which may contribute to alterations in endogenous estrogen production are needed. The acquisition of data under this contract is critical to understanding the degree of decline in estrogen production and the alternative methods by which estrogen production occurs in postmenopausal women. Also, a rational approach to replacement therapy in postmenopausal women cannot be developed until quality and quantity of endogenous estrogens produced during the menopause are clearly delineated.

Proposed Course: The contract is to continue for a minimum of three years.

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Adult Development and Aging Branch
Contract and Collaborative Research

Contract Number: 72-2721

Contract Title: Production Colony of Aging Rats in an Isolator Environment

Contractor: Charles River Breeding Laboratories, Wilmington, Massachusetts

Money Allocated: $10,942

Objectives: 1. Establish a production, rearing and maintenance system for aging rats that prevents introduction of pathogens prematurely shortening the natural lifespan of aging laboratory rats.

2. Determine the commercial feasibility and per animal cost effectiveness of isolator rearing of aging laboratory rats compared with barrier and conventionally reared rats.

3. To develop for research in aging:

a) a limited source of aging rats free of bacterial and detectable viral diseases.

b) a system of environmental control that is standardized and transferable to the research laboratory without unduly compromising the biological integrity of the animals.

c) laboratory rats that are known to survive to natural senescence as a natural population independent of bacterial diseases or detectable viral diseases.

4. Compare survival, pathology and degenerative change in isolator reared rats with barrier and conventionally reared animals.

Significance for Aging Research: The lack of aging experimental animals of defined quality is one of the major limiting factors to the study of aging, particularly animals that survive to natural senescence. Until recently aging studies in animals, especially rodents, were limited to those animals hardy enough to survive the stress of disease and a fluctuating physical environment. During the past several years several methods have evolved in the development, husbandry, maintenance and care of laboratory animals that permit routine cesarean derivation and rearing of laboratory rats and other experimental animals behind a barrier (SPF) or in the rigidly controlled environment of plastic isolators. The isolator excludes the introduction of bacterial and detectable viral agents. This methodology combined with genetically defined animals on a stabilized diet and a close monitoring system goes far toward establishing and providing aging animals that survive to natural senescence independent of the complications of infectious disease, parasitism, or wide variability in physical environment.

Basically the contract for an isolator reared colony of aging rats is concerned with developing an aging rat colony in a rigidly controlled isolator environment free of bacterial agents, except those purposefully introduced as normal bacterial flora. Changes in the aging process can most readily be determined when environmental conditions are uniformly controlled and sufficient numbers of animals representative of the total population of animals survive to an aged condition that can be studied as characteristic of the normal processes of aging. For this reason the contract requires uniformity in environment, diet, humidity and temperature and genetic quality of the animals in the isolator.

Once it is clearly established that laboratory rats can be maintained for their full lifespan independent of detectable microbial disease, and survival rates largely offset added costs for isolator maintenance, a resource of isolator reared animals can be provided to investigators in aging. The need for developing expensive and sophisticated environmentally controlled buildings to support the colony is unnecessary since the total environment of the animal is transferred within the isolator unit.

Studies of aging independent of environmental variables and disease can then be conducted to increase understanding of aging processes in animals and how these processes may apply to man or lead to experimentation in man. The major significance of the isolator system for rearing laboratory animals lies in the enhancement of detectable change in aging processes.

Proposed Course: The isolator contract is to be continued for a minimum of two years. Continuation will be dependent on commercial feasibility and colony survival rates.

ADULT DEVELOPMENT AND AGING BRANCH
CONTRACT AND COLLABORATIVE RESEARCH

CONTRACT NUMBER: Unassigned

CONTRACT TITLE: Review, Analysis and Evaluation of population Models

CONTRACTOR: Duke University, Durham, North Carolina
(Contract Officer: Dr. George Myers)
(Subcontract to Research Triangle Institute)

MONEY ALLOCATED: $80,000

OBJECTIVES: This contract supports the review and critical evaluation of models and techniques for projecting future population trends. The objective is to provide a basis for the design and specification of a computerized mathematical model for projecting the future size and structure of the middle-aged and elderly population.

Mortality changes are often a neglected aspect of projection procedures and appropriate attention will be given to developments in this field. Considerable attention will be given to the notion of competing risks of death in a mathematical sense and models that can readily incorporate this element into various projective procedures.

SIGNIFICANCE FOR AGING RESEARCH: The information obtained from work supported by this contract will be important in planning future health research and services for the middle-aged and elderly.

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Mental Retardation Branch

The Mental Retardation Branch has primary responsibility for research in the biological, behavioral and social aspects of mental retardation within the context of child health and human development. This responsibility embraces all factors--biological, social and behavioral--that influence subnormal development.

The greatest hope for mastery of the problem of mental retardation lies in its prevention, or when this is not possible, in the promotion of the individual's maximum skills through a wide range of habilitative and restorative procedures. The goals of the Mental Retardation Branch then, are the facilitation of research directed toward the identification, prevention, and amelioration of mental retardation.

Research Activities:

Institute support for research and research training directly related to mental retardation increased sharply during fiscal year 1972 continuing the pattern of rapidly increasing support evidenced during the previous year. As of January, 1972 the Institute was supporting 92 research grants, totaling approximately $12.4 million. This represents an increase of approximately $2.3 million in research grant support since January of 1971. Similar growth occurred in the program of directed research supported by contracts. Institute support for research training, fellowships, and research career development awards totaled approximately $1.7 million, an increase of $200,000 over FY 1971.

The research supported covers a broad range from almost every branch of the physical, biological, psychological, social and clinical sciences. These investigations are concerned with the etiology, epidemiology, pathophysiology, diagnosis, prevention and amelioration of mental retardation. Program project research grants involving team efforts directed toward solution of major mental retardation problems are strongly represented in the Branch program of research. These large and complex grants are concerned with both biomedical and behavioral research issues and tend to emphasize research on problems at the interface between disciplines. A major share of Branch research activity is concentrated in the Mental Retardation Research Centers constructed and staffed for the purpose of conducting a major research attack on the problem of mental retardation. Details of some of the research activities supported by the Branch follow.

Continuing research on PKU provides important information on the efficacy of dietary therapy. In a large scale study of 82 PKU children it was noted that therapy must begin within the first 3 weeks of life to prevent intellectual retardation. After treatment for an average of 50 months (36-60 months), children taken off the diet for 2 to 6 years showed no evidence of developmental deterioration. Further research and assessment of this important observation is underway in other laboratories to aid our under standing of dietary therapy.

The concentrated study of inborn errors of metabolism, in addition to the already well known investigations and partial remedies for phenylketonuria and galactosemia, has yielded significant and possibly unexpected results. One investigator, for example, has discovered that kidney transplantation may provide a possible means of controlling Fabry's disease and other inborn errors. This disease is a glycolipid storage disorder primarily affecting the kidneys and ultimately resulting in death through renal failure. The principle of organ transplantation to supply missing or inactive enzymes may have useful application where the blood-brain barrier is not involved.

Studies on intrauterine undernutrition and the nutritional status of migrant children lend further support to the role of this factor in mental development. The analyses of brains from human infants who died at birth and who were small in weight for gestation, indicate significant brain alterations and point to the need for intensive postnatal care of such infants. The major nutritional problem among the migrant children was Vitamin A deficiency which correlated statistically with a high level of skin and upper respiratory infections. Other protein and vitamin deficiencies and elevated cholesterol levels were also found. This latter finding may have relevance to adult disease conditions.

The role of protein metabolism in brain growth is being studied in several ways. A center investigator has found that there are three hormones without which neural outgrowth cannot take place. These same hormones are essential for protein synthesis and polysome formation. In a related study, protein deficient female rats gave birth to litters having a reduced number of cortical neurons. These effects are transgenerational; even if the first generation offspring are properly fed, the second generation continues to have fewer neurons and a higher rate of mortality and morbidity.

The early identification of children with handicapping conditions is an important precursor to effective treatment. One Institute supported investigator has developed a screening instrument to be applied by health aides from poverty areas. After training, these aides in Denver have now screened 10,000 preschool aged children. The technique is being set up in other cities and states while additional, more simplified tools are being constructed. This activity is a small part of a far-reaching program of research directed toward detection and refined diagnostic procedures.

Several types of mental subnormality are associated with chromosomal abnormalities. Significant progress has been made in cytogenetic research of relevance to these disorders. An Institute-supported investigator has

recently devised a simple, quick, and accurate method to identify each human chromosome. This important finding will make the diagnosis of chromosomal defects more precise and is particularly significant at this time when increased demands are being made for genetic counseling.

Considerable progress is being made at one of the centers on a large group of genetically determined diseases characterized by the storage and excretion in tissues of mucopolysaccharides. The discovery that fibroblasts cultured from skin biopsies of affected individuals display the metabolic abnormality of these diseases has made possible the intensive study of mucopolysaccharidoses. Since this group of conditions is characterized by the excretion of a chemical in the urine one can look for the presence of this substance in cell-free amniotic fluid. The rationale is that amniotic fluid is composed, in part, of fetal urine. Center investigators demonstrated an abnormal polysaccharide pattern in amniotic fluid obtained at the 14th week of pregnancy. There was a fourfold increase in the amount of mucopolysaccharide in the amniotic fluid compared to normal controls. The infant was delivered and was found to have Hurler's syndrome. In another patient, who was also at risk to develop Hurler's syndrome, amniotic fluid obtained during the 16th week of pregnancy showed no abnormality. Amniocentesis was repeated at 22 weeks of gestation and was found to have abnormal quantities of sulfated polysaccharides. The liver of the aborted fetus confirmed the diagnosis in utero.

These data indicate that an abnormal polysaccharide pattern can be detected in amniotic fluid. The optimum time for amniocentesis remains to be determined. These investigators are continuing to compare resulta of chemical analyses with those based on study of cultured amniotic cells. The former test can be completed within two days while the latter is dependent upon the establishment of tissue culture lines which could take a number of weeks and is non-specific. Early prenatal diagnosis is important especially in those states which prohibit therapeutic abortion after a certain gestational age is reached.

Research center investigators at Children's Hospital Medical Center, Boston, have determined that mild brain edema produced by experimental seizures can be prevented by dexamethasone. The occasional brain damaging effect of prolonged status epilepticus is well known, and this experimental model in the animal indicates that sustained seizures produce local breakdown in the blood-brain barrier accompanied by an increase in brain water. The latter can be completely prevented by dexamethone, and the permeability changes greatly reduced. This may be a lead in the understanding and prevention of some causes of mental retardation in the seizure patient.

The effects of life circumstances on the stability of intelligence has been studied by investigators at the research center, Albert Einstein College of Medicine, Bronx, N. Y. These investigators found no evidence for deterioration of IQ with age in disadvantaged children tested at three and at six. Earlier studies have suggested that children from disadvantaged backgrounds show a decline of measured intelligence with age.

Mental Retardation Research Centers:

The MRRC construction grant program was authorized under P.L. 88-164 to conduct research in mental retardation and related aspects of human development. Twelve centers have been created under this program, all of them located on, or closely affiliated with a university. Most have close working relationships with residential or outpatient diagnostic facilities for the retarded for research and training purposes. All but one of the centers - the University of Wisconsin - have completed construction and occupancy.

The research center program, now approximating full operational status, represents the major research thrust of this nation to elucidate the causes and pathogenesis of retardation and to develop methods for its prevention, treatment and amelioration. The complexity of the symptom and its multiple and diverse causes demands the talents of many biological and behavioral scientists in independent and collaborative research activities. The centers, by virtue of their multidepartmental composition are uniquely equipped to implement the interdisciplinary research model.

The NICHD has primary program and administrative responsibility at the Federal level for the center program. To insure the long range viability and stability of center research programs, the Institute adopted early, a program of core support for administrative and supportive services, common equipment and supplies and new program development personnel. The latter support category, though time limited to maintain quality controls, has proven most meaningful in making these resources centers of excellence. It has enabled the centers to recruit outstanding scientists and develop new research areas, and compete successfully for program project and individual grant support from funding agencies.

The research and training activities of the centers are highly varied. Some are heavily concentrated on basic, clinical and applied aspects of the biomedical sciences (Colorado, Chicago, Cincinnati); another in the behavioral sciences (Peabody), while the large majority have significant efforts in both the biological and behavioral sciences, albeit with differin degrees of emphases. As might be anticipated, several centers are directing their research efforts toward similar major issues and causes of mental retardation.

An important shared concern of center scientists is the heavy prevalence of mild retardation among socioeconomically disadvantaged populations and the correlates of poverty that appear associated with low intellectual performance. Much of the ongoing research in this area has potential for ready application. Different dimensions of the problem are being investigated. Thus, ongoing studies on animals and humans at UCLA, Colorado and Einstein College of Medicine are further defining the role of malnutrition during critical periods of brain growth and development and the possibilities of controlled intervention. The interactional effect of malnutrition with deficits in social stimulation are also being studied with interesting results.

Efforts to improve intellectual and social performance through environmental enrichment and early stimulation programs for young infants and preschoolers have been initiated on a significant scale at a number of centers. These programs include innovations in language training, motivational systems, classroom behavior, teaching technologies and modification of parental behavior and child rearing practices. Most of these research efforts are being applied in naturalistic community settings and thus may find ready acceptance by school and community leaders. A wide spectrum of basic behavioral research activities in cognition, communication style, language acquisition and development, perceptual and motor skills and social and health correlates of learning disability undergird these intervention projects.

Professionals in mental retardation have long recognized the importance of early detection and diagnosis in preventing mental retardation and its secondary handicaps. Epidemiological studies that can specify factors associated with this symptom are very useful in identifying groups at risk, what conditions need change and where intervention strategies should be applied. In one center the impact of programmed stimulation and handling of very young infants born prematurely or otherwise at high risk, is being investigated. The epidemiologic method, in conjunction with other inputs from collaborating investigators is being used to study: populations for prevalence of developmental abnormalities; population mobility in institutions and community facilities; effects of malnutrition; impact of alternative patterns of care on the behavior and development of retarded persons.

Technological advances in prenatal diagnostic procedures, together with new discoveries in biochemical and genetic correlates of mental retardation are expanding preventive potentials. The centers, because of their prime concern with developmental defects, are in the forefront of investigations in this area. As the application of amniocentesis procedures spreads throughout the country and more laboratories become involved in biochemical assays and tissue culturing, the need for standardization and other safeguards is becoming increasingly apparent. Center researchers are actively pursuing these interests, are exploring other technologies for the detection of defects in utero, and are seeking alternatives to therapeutic abortion as a preventive method.

The research centers have a clear mission to prevent retardation or ameliorate its effects on the individual, his family and community. These highly practical goals however, cannot be achieved without the substrate of fundamental knowledge of the biology and behavior of the human organism, normal or aberrant. Thus, a meaningful proportion of center resources is devoted to areas of basic research such as those involving neural tissues, embryonic development, genetic mechanisms, cell growth, transport phenomena and fetal endocrinology. While such studies have less immediate relevance to the problem of retardation, the knowledge to be derived therefrom, when coupled with clinical experiments, formsthe bridges to ultimate prevention and treatment.

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Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: NO1 92180
Contract Title: Prevention of Prematurity and Developmental Disorders Through Nutritional Supplementation and Laboratory Studies on Prenatal Malnutrition
Contractor: Columbia University School of Public Health
Money Allocated: $470,000, FY 72
$123,000, FY 72

Objective: The purpose of this contract is to carry out a controlled study on the effects of nutritional supplements to pregnant women in the socioeconomically deprived population of Harlem in New York City. Evaluation will be made of the impact of these supplements during various stages of pregnancy on birth weight, infant development, infant mortality and related disorders. Subjects will be drawn from a clinic population considered at high risk of giving birth to premature infants. This project is presumed to have great social and practical significance for improving physical and intellectual development in racial minority and poverty groups and for the prevention of mental retardation and related handicapping conditions.

The laboratory studies are designed to obtain a better understanding of the physiological parameters of growth and development and the potential role of infectious processes in low birth weight and retarded development. The correlation of data from these studies with pathological and clinical findings and data derived from the basic project will strengthen and extend the results and validity of the nutritional experiment.

Methodology: Experimental and control groups will be selected from a clinic population serving the Harlem area according to certain indices of high risk in producing low birth weight infants such as prepregnancy weight, previous history of prematurity, weight gain during pregnancy and age of mother and low protein intake. Other relevant factors including smoking habits, drug usage and trimester of entry into the project will be considered in the analysis. Infants will be assessed developmentally at birth, 26 and 40 weeks of age.

The laboratory studies include biochemical measurements of DNA, RNA and protein on placental and autopsy material and their relation to cell size and number. Morphometric measures will be made on by-products of pregnancy and infant tissues and studies of infection will be undertaken on maternal and infant serum and cultures.

Results: The project is now entering its 3rd year and has completed its first full year of subject enrollment. To date, approximately 470 women are active participants in the project and about 250 babies have been delivered. An anticipated 20 more months will be required to complete the proposed enrollment of 1200 women. Recruitment rates have been highly satisfactory and attrition has been low. No analysis has been made as yet of the outcomes of this study.

The supplementary laboratory work was not initiated until February, 1972.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: NIH-2447

Contract Title : Diagnostic and Intervention Studies of High-Risk Infants

Contractor : Regents of the University of California

Money Allocated: $441,225, FY 71

Objective : The purpose of this contract is: (a) to develop new diagnostic techniques and relate these to established ones, so as to best delineate those aspects of psychophysiological functioning in high-risk infants that indicate continuing risk for development, and (b) to ameliorate or modify this risk status through a program of specified interventions implemented from four or nine to twenty-four months of age.

Methodology : This prospective study is designed to specify those behaviors of high-risk neonates and infants which relate to performance on tests at two years of age. The study design is longitudinal, and correctional, covering a two-year period in each child's development.

The study sample of 200 subjects will be drawn from the UCLA Newborn Nursery and will consist of all premature infants of 37 weeks gestational age or less. The control subjects, full-term infants (39-41 weeks gestation) will also be drawn from the UCLA Newborn Nursery.

In the newborn period the infants will be rated on several behavioral and physiological diagnostic tests as well as on obstetrical and neonatal events. In addition, the infant's performance on other diagnostic tests will be assessed during the first four months. All infants and their parents will be provided with intensive medical, nursing, and social work support and anticipatory guidance in the nursery, well-baby clinic, and in the home.

When the infant is four months of age, the scores on all measures will be weighted and summed to derive a 4-Month Cumulative Risk Score (CRS). The CRS will then be used to classify the four-month old subjects as high or low risk. The infant will then be placed into one of the experimental or control groups. A randomly selected group of high-risk infants will begin to receive specialized 4 Month Intervention.

Additional diagnostic tests on behavioral and physiological measures will be administered during the four to nine months period. Medical, nursing, and social work support will continue for all babies both in the well-baby clinic and in the home. Scores on all of the diagnostic tests will be weighted and summed to yield a 9-month CRS. The 9-month CRS will be used to validate the risk and non-risk status of the 4-month CRS. At nine months of age, half of the high-risk-nonintervention group will receive 9-month specialized intervention. Intervention will continue with these infants and with the original HR-4-month specialized intervention group until the infants are two years of age. The diagnostic measures and the specialized intervention program, including age of intervention, will be evaluated by testing each child at two years of age on a variety of measures.

Results : The project is in its first year of operation. During this period staff has been recruited, study facilities developed and organized, the research program planned, and pilot studies conducted.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: NIH 71-2446
Contract Title : An Investigation of Certain Relationships Between Hearing Impairment and Language Disability
Contractor: University of Washington, Seattle, Washington
Money Allocated: $166,359, FY 71

Objective: The three specific objectives of this contract are: (1) to determine the feasibility of conducting an extensive investigation of the relationships between hearing impairment and the speech and language status of children from birth to three years of age; (2) to formulate a test battery for assessing the speech, hearing and language abilities of children under the age of 36 months; and (3) to develop specific testable hypotheses regarding the relationships between hearing impairment and language function. The results of the two year contract will serve as a base for a subsequent project on the investigation of the relationships between hearing impairment and language in young children with both mental or physical impairment and with other problems, as cultural, familial, mental retardation, physically based mental retardation, and cerebral palsy.

Methodology: The research will be conducted in four major areas: (1) auditory perception, (2) receptive and expressive language, (3) speech production, and (4) communicative interaction. In the area of auditory perception four facets will be examined: (1) behavioral responses to different auditory stimuli will be examined utilizing a variety of play techniques and conjugately programmed stimuli with a specially constructed manipulanda; (2) speech sound discrimination of 2-3 year olds will be tested using a key-foil discrimination task; (3) auditory sensitivity, examining behavioral responses using a population of 6-24 month old infants; and (4) auditory sensitivity, temporal integration and discrimination between paired auditory stimuli will be examined in infants using modifications of averaged electroencephalic audiometry.

Receptive and expressive language will be examined using the Sequenced Inventory of Language Development. In addition, phonological, morphological and syntactic development will be studied by transcribing language samples recorded on video tape. Speech production will be studied by (1) describing the vocalizations of infants in terms of feature networks, (2) describing the prosodic skill development in both normal and hearing impaired children under the age of two, and (3) measuring such physiological aspects of speech control as lip movement, jaw movement and lung volume. Communicative interaction will be examined using video tapes obtained in the child's home and evaluated for communicative and interactive behaviors. The tapes will be studied for number of vocalization-verbalization behaviors, communicative events, antecedent events, consequential events and behavioral events.

Results: This project is in its first year of operation. During this period staff has been recruited, equipment has been obtained, and several pilot studies conducted.

NICHD ANNUAL REPORT
July 1, 1971 through July 30, 1972
Mental Retardation Branch
Collaborative Research and Contracts
Summary

Contract Number: NICHD 72-2016
Contract Title: The Development of Materials for Application of Behavior Modification Skills by Parents of Mentally Retarded Children
Contractor: Behavior Education Projects, Inc.
Money Allocated: $92,459, FY 72

Objectives: The purpose of this contract is to test the efficacy of printed materials as used by parents, in shaping the behavior of their retarded children. Determination will also be made of the degree of supplementary training which may be necessary in the useof such materials and the relationships between child and parental characteristics and training effectiveness.

Methodology: This project involves several phases of operation. Initially, parents will identify problems and training needs posed by the behavior of their children and programmed texts will be developed responsive to these needs. After these materials have been pretested, controlled experiments will be undertaken varying the use of the texts and degrees and form of supplementary training. Parent and child characteristics will be included in the analysis of the data.

Results: This project was initiated in October, 1971. To date, several programmed texts in the area of self care skills have been developed and the participation of parent groups and preschool programs have been secured. The experimental phase of the project will be initiated concurrent with the development of additional printed materials.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Mental Retardation Branch
Collaborative Research and Contracts
Summary Reports

Contract Number: NICHD-71-2081
Contract Title: Intellectual and Behavioral Consequences of Severe Malnutrition in Infancy: A Collaborative Study
Contractor: Albert Einstein College of Medicine
Money Allocated: $66,720, FY 72

Objectives: The aims of this study are to assess the relationship between severe malnutrition during the first two years of life in children and their subsequent intellectual development and behavior. Control groups of siblings and classmates are being compared on variables related to somatic, neurological, phsyiological, intellectual, educational and family characteristics.

Methodology: This is a retrospective study of children aged 6-10 years who were hospitalized for severe malnutrition before two years of age. Detailed clinical records were kept of these infants regarding clinical severity of illness, duration of stay and somatic measures. In the follow-up study information on the index children and controls was obtained on a wide range of developmental and behavioral indices, school behavior and achievement, child rearing and social environmental stimulation.

Results: This project has now completed the first year of a two year grant for data analysis. Seven papers have been published or are in press. Findings thus far indicate that the index children are shorter, weigh less, have smaller head circumference, lower IQ's, lower school achievement and less satisfactory behavior in school. Malnutrition and lack of social stimulation both appear to play contributory roles. The age of hospitalization did not appear significant in affecting developmental outcomes.

NICHD Annual Report
July 1, 1971 through June 30, 1972
Office of Associate Director for Program Services
Extramural Activities

SUMMARY

One of the major impacts on the Office of Program Services during Fiscal Year 1972 was the transfer of the contracting authority from the Office of Grants and Contracts, Office of the Associate Director for Administration, OD/NIH, to the Office of Program Services' Research and Contracts Management Branch. A new contract section in the Grants and Contracts Branch was established by combining staff already in the Branch plus staff transferred from the Office of Contracts and Grants. The new contract section has a total of 16 persons. It is expected that 284 contracts and reimbursable agreements valued at about $21 million will be processed by the section during this fiscal year. At the same time there has been a large increase in the number of research and research training grants, research fellowships, and research career development awards handled by the Branch. Together with research contracts they total approximately $95,000,000. This represents about 15% increase in the number of projects (190) and about 25% in dollars ($18.7 million) from last year's portfolio. Also of major importance is the fact that the number of NICHD research grant applications submitted for review during the fiscal year has reached an all time high of 1,280 applications in the amount of $72.2 million. However, during the year only about 32% of the number and 25% of the dollar value were funded. The Institute ranked first or second among all the other NIH Institutes and Divisions in each Council meeting during the year in the number of new research applications reviewed.

This large increase in applications imposed an additional workload on most units in the several branches of the Office of Program Services.

Competing research grants except in the Population program were negotiated at an average rate of 6%. Through these negotiations it is expected that an additional 13 grants will be funded. All training grants were negotiated at the same average (6%) which should result in an additional 8 training grants being funded.

Our research grant program continued to increase in 1972. We expect to award 867 regular research grants (an increase of 119 over 1971) in the amount of $54.5 (an increase of $12.2 over 1971).

In our training grant program we expect to award 120 grants for $10 million, an increase of 8 grants but no increase in dollars over Fiscal Year 1971.

The Program Statistics and Analysis Branch was reorganized shortly before the beginning of the current fiscal year and under their new responsibilities participated in the publication of the inventory of Population Research and the development of a more sophisticated and expanded computer file for use as a data base for Branch studies and other information needs of the Institute. Members of the Branch worked closely with the staff of the

Center for Population Research in the development of their expanded program while continuing to provide statistical and analytical material to meet the many demands of the Institute including special reports to the Institute's National Advisory Child Health and Human Development Council. The Branch's Program and Analysis Section and Statistical Analysis Section provided a great deal of material to support the development of the five program areas of the Institute.

The Scientific Conference Branch provided major assistance in the planning and arranging for seventeen conferences directly sponsored by NICHD. Approximately 543 scientists participated in these meetings. Institute conferences have always been a major vehicle for developing the variety of programs of the Institute. The Branch is also responsible for the committee management functions for the Institute. The Institute currently has eight advisory committees including the National Advisory Child Health and Human Development Council. The Council has expanded its membership to fourteen. The Branch Chief, as Council Secretary, has been responsible for assisting in implementing the increased Council membership as well as the Action Plan for the Appointment of Women to Advisory Committees.

The Office of Program Services continued to stress the importance of staff development. A large number of staff members from all organization units participated in some form of training. We also continued to emphasize EEO activities which included two half day discussion sessions in which all members of the Office of Program Services participated.

Emphasis was also given to continued staff communication through weekly meetings of staff groups at several organizational levels.

The physical separation of the Scientific Conference Branch and Program Statistics and Analysis Branch in the Westwood Building and the Contracts Section of the Grants and Contracts Management Branch in the B Wing of Building 31 continues to create problems of working relationship with the remaining staff in the A Wing of Building 31. The effectiveness of staff working relationship on a face-to-face basis is seriously curtailed.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Grants and Contracts Management Branch

SUMMARY

During Fiscal Year 1972, the Institute should support about 1,501 research and research training grants, research fellowships, research career and research career development awards and research contracts at approximately $95.0 million. This would represent an increase of 15% in numbers of projects (190) and an increase of 25% in dollars ($18.7 million) from the Fiscal Year 1971 portfolio.

The Institute used a decile system as the basis for funding research grants competing at the June 1971 Council. For the November 1971 and March 1972 Councils, the DRG system of normalized scores was used to determine a funding order. Program projects were treated differently however, using an Institute developed system of normalized scores.

Training grants were funded on a priority score basis during Fiscal Year 1972. The priority score was essentially the basis for funding the research fellowship career award, and research contract programs, although selected adjustments in funding order were permitted because of program relevance.

Negotiation of Research and Training Grants

Noncompeting research grants were not negotiated during Fiscal Year 1972. Competing research grants, in programs other than Population, were negotiated at an average rate of 6%. Through these negotiations we expect to acquire an additional $0.8 million which will enable us to fund about 13 additional grants. All training grants were negotiated at an average rate of 6%. The negotiations will result in a savings of approximately $0.6 million which will enable us to fund 8 additional training grants.

Research Grants

During Fiscal Year 1972, we expect to award 867 regular research grants in the amount of $54.5 million, an increase of 119 grants and $12.2 million over Fiscal Year 1971. The total consists of approximately 520 noncompeting grants for $31.7 million and 347 competing awards for $22.8 million. In our Special Research Program we will award 11 Mental Retardation Center grants for $5.6 million. We transferred $3.9 million to the Division of Research and Resources for the General Research Support Grant Program. We will award $0.1 million for scientific evaluation grants.

Training Grants

During Fiscal Year 1972, we expect to award 120 training grants for $10.0 million, an increase of 8 grants and no increase in dollars over Fiscal Year 1971. The total consists of 79 noncompeting grants for $7.3 million and 41 competing grants for $2.7 million. We will obligate all of the $0.1 million for scientific evaluation grants.

Fellowships

We expect to award approximately 115 postdoctoral and special fellowships in the amount of $1.0 million. This is an increase of 15 awards and $0.2 million from Fiscal Year 1971.

Research Career Awards and Research Career Development Awards

During Fiscal Year 1972, we expect to award 113 research career and research career development awards in the amount of $2.6 million. The total consists of 7 research career awards for $0.2 million and 106 research career development awards for $2.4 million. This represents a decrease of 7 awards and $0.1 million over Fiscal Year 1971.

Research Contracts

During Fiscal Year 1972, we expect to process 284 contracts and reimbursable agreements for approximately $20.9 million. This will consist of approximately 210 research contracts and reimbursable agreements in the amount of $15.8 million for the Center for Population Research Program, about 29 contracts in the amount of $0.9 million in the Adult Development and Aging Program, and approximately 45 research contracts in the amount of $4.2 million for the Child Health Program Areas.

The Fiscal Year 1972 contract program will represent a net increase of 64 contracts and reimbursable agreements and $7.6 million over the Fiscal Year 1971 program.

SUMMARY OF INSTITUTES SUPPORTED PROJECTS
(Dollars in Millions)

	1971		1972		Change	
	No.	Amount	No.	Amount	No.	Amount
Research Grants						
Noncompeting	541	32.5	529	36.2	-12	+ 3.7
Competing	218	13.8	350	24.2	+ 132	10.4
	759	46.3	879	60.4	+ 120	+ 14.1
Training Grants						
Noncompeting	90	7.3	79	7.3	-11	0
Competing	22	2.7	41	2.7	+ 19	0
Scientific Eval.	0	.1	0	.1	0	0
	112	10.1	120	10.1	+ 8	0
Research Fellowships	100	.9	105	1.0	+ 5	+ 0.1
Research Career and Research Career Development Awards	120	2.9	113	2.6	- 7	- .3
Contracts	220	16.1	284	20.9	+ 64	+ 4.8
Grand Total	1,311	$76.3	1,501	$95.0	+ 190	$ 18.7

NICHD Annual Report
July 1, 1971 through June 30, 1972
Program Statistics and Analysis Branch

FY 1972 was the first full year the Branch functioned under its reorganized status. Its major new responsibilities included the summarization of progress in research supported by NICHD, the publication of inventories of population research, and the development of a more sophisticated and expanded computer file for use as a data base for Branch studies and other information needs of the Institute.

In connection with the improvement of communication of information in the field of population research, the Branch worked closely with staff of the Center for Population Research and with the Interagency Committee on Population Research. The Chief of the Branch was asked to serve on a number of subcommittees and task forces of ICPR, including those on the development of an improved information system in population research, the improvement of data collection and analysis in the biological and social sciences, data generation activities, and the ICPR annual narrative report to the Deputy Assistant Secretary for Population Affairs.

In addition to studies and reports prepared for the NACHD Council and for program staff, the Branch produced two publications for distribution to the scientific community: "Population Research, the Federal Program" and "The Extramural Program of Research on Aging."

To improve their effectiveness in the work of the Branch, and to provide opportunities for self-improvement and advancement, most of the Branch staff participated in training activities. Courses taken included statistics and operations research, remote terminal operations and advanced programming, developmental biology and endocrinology, technical and report writing, and shorthand and other office skills. In addition, several staff members are attending the Upward Mobility College, and one is working toward a high-school equivalency certificate.

Staff of the Branch are cooperating in EEO activities on an informal basis, and two staff members are more formally involved as members of the NICHD EEO Advisory Committee. One of these is also editor of the NICHD EEO Bulletin; in addition, the other is an NIH Counselor and a member of the NIH EEO Council.

Program Analysis Section

Major functions of the Program Analysis Section continue to include the assignment of grant applications to appropriate program areas in the Institute and to categories within these program areas, the scientific classification of grants and contracts, and the preparation of analytical reports in research areas pertinent to the interests of NICHD.

An undertaking which has taken up a large portion of time in the Section is the preparation of the FY 1971 inventory of federally supported research in population. Revisions were made in the questionnaire sent out to the various

agencies in order to clarify the specific items of information required. A great effort was made this year to obtain complete information on all projects submitted, and the material was then entered on a computer file, which should simplify the preparation of future reports. Emendations were added to project titles where indicated, and major changes were made in the format of the publication. The introduction included, for the first time, a brief summary and analysis of the data in the inventory.

Another publication for which the Section has responsibility is that describing the extramural program of the Adult Development and Aging Branch. Summaries prepared for each project supported from FY 1971 funds were mailed to the individual investigators for review prior to use in the publication. A new format was used for the summaries this year, which it was hoped would provide more informative detail to the reader on the kinds of research on aging supported by the Institute.

Several requests received by the Section resulted from specific inquiries in Congress. One of these was concerned with abortion, and we were asked to provide a description of NICHD projects dealing with both therapeutic and spontaneous abortion.

Another request initiated by Congressional interest was in an area which has received considerable mention in the press; namely, the Sudden Infant Death Syndrome. One of the difficulties in defining research in this area has been the lack of positive findings concerning its etiology. Although NICHD supports only a few projects specifically concerned with SIDS, there are quite a number in other areas which could provide relevant information on the etiology of this syndrome, and, in consultation with program staff, these areas were defined and the appropriate projects identified.

Other requests were generated by program staff in relation to the development of new foci of emphasis within the Institute or to the expansion of areas in which research is already being supported by the Institute. One of these in which considerable interest has been expressed is pediatric pharmacology in the pre- and postnatal periods. As background material for a meeting on this subject, a narrative analysis was prepared of the Institute's holdings in this area. This material was also distributed to members of the NACHD Council.

One of the research areas which has been greatly neglected is that of the effects of the man-made environment on the developing child. There has been little communication, for example, between architects designing classrooms and behavioral scientists interested in the children who will occupy these rooms. An analysis prepared by the Section pointed out that NICHD supports very few projects specifically in this field, and indicated the kinds of research currently supported which might be extended to include variables in this area.

There has been considerable emphasis in recent years on research dealing with early childhood. Staff of the Growth and Development Branch are now interested in developing a program focused on adolescence. A series of conferences is being planned, and as general background for these and for members of the NACHD Council, the Section was asked to prepare a narrative describing research currently supported by NICHD in this area.

A great many other requests for information on projects in a wide variety of research areas were answered. Among these were the following: breast feeding, sex predetermination, mongolism, neurosurgery, graphic analysis by computer, genetics, hyperoxygenation in the aged, and nutrition.

The Chief of the Section serves as an NICHD member of the Interagency Panel on Early Childhood Research and Development, and the staff has worked with the Panel to provide them with information on NICHD research, and to help them improve their methods of data gathering.

Research Analysis Section

The Research Analysis Section was established about a year ago as a result of a reorganization of the functions of two of the Branches in Program Services. Staff losses and difficulties in recruiting delayed the implementation of the Section's new functions, but with the acquirement of a Section Chief, it became possible to focus major effort on the preparation of summaries of research progress in the five program areas. Special emphasis has been given to aging grants, and to nutrition studies, both primary and subsidiary. Still in progress are efforts to devise a procedure for summarizing the yearly activities of program projects and center grants.

This work represented the basic ongoing activities of the Section, but staff also worked with other Sections on a number of special assignments. Examples of these include an analysis of thesis titles of trainees in the mental retardation program receiving direct or indirect support under NICHD training grants, preparation of material for the HEW publication "Mental Retardation Grants," types of drugs and agents used in NICHD pharmacology - relevant studies, an estimate of the average number of individual studies and PI's represented in program projects, and the scientific classification of NICHD grants.

Statistical Analysis Section

FY 1972 was characterized by an increase in the demands made on the Statistical Analysis Section for special reports, analyses, and studies. More special requests were processed in the first nine months of FY 72 than in all FY 71. On the average, the Section worked on 15 special jobs per month in FY 72 compared to 11 special jobs per month in FY 71. These special requests originated both within and without the Institute. The largest share, 30 percent, came from the Office of the Institute Director, including the Budget Office, Office of Program Planning and Evaluation, and the Office of Public Information. The second largest share of special projects (28 percent) was processed on behalf of the Associate Director for Extramural Programs and the staff of the Extramural branches. Requests from within the Office of Program Services accounted for 16 percent of the special projects and from the Center for Population Research, 12 percent. The remaining projects represented instances in which the Section was able to assist offices in NIH, in DHEW, in other Government agencies, and in the private sector.

Some of the projects investigated the fate of grant applications and examined approval and funding rates. Other reports showed the distribution of grants and contracts by categories, such as academic departments, domestic/foreign

location, program subclasses, etc. One study performed was a detailed analysis of the review actions of a particular review group. In another, the Section found a significant relationship between raw priority scores and the proportion of budgeted salaries to total budget in a sample of research grant applications. The Section also provided cost estimates of individual intramural projects. As needed, the Section secured relevant vital statistics from the National Center for Health Statistics for staff use, providing interpretations where desirable. These examples illustrate the kinds of data requirements which the Section filled.

Certain studies were prepared especially for presentation to the National Advisory Child Health and Human Development Council. These included a trend study of program projects and center grants in the Institute, a series of display charts showing the growth in the numbers of grant applications assigned to NICHD, a trend analysis of the fate of grant applications in the Adult Development and Aging Branch, and another series of display charts depicting significant trends and a variety of important factors in NICHD grant and contract programs. Still another report, in process, provides a look at trends in training grant trainee support by program area, by academic level, by degree sought, etc.

The Section, as in previous years, was responsible for submitting the Institute's annual report to the Secretary's Committee on Mental Retardation covering research and research training in mental retardation. The NICHD Section of the NIH annual report to the National Science Foundation was also a responsibility of the Section.

Recurring reports on NICHD grant and contract holdings, both on a fiscal year basis and for active grants, continued to be published by the Section for the use of Institute staff.

Table 1 on page M6 summarizes the Institute's portfolio of grants and contracts active during March 1972. Table 2 (page M7) provides number and dollar totals of NICHD grants and contracts by mechanism of support for the period FY 1964 through FY 1971. The decline in the number of research grants supported continued in FY 1971 even though grant funds available increased. This reflects larger average grant size brought about not only by inflationary growth in grant budgets and increases in indirect cost rates, but also by an upward trend in the number of program projects and center grants in the Institute's portfolio. Research contracts jumped from 143 awarded in FY 1970 to 221 in 1971, and contract funds more than doubled to $16.1 million, reflecting principally the contract program of the Center for Population Research.

Information Systems Section

The Information Systems Section was set up as a new Section within PSAB at the time the Branch was reorganized. During the transition period following the reorganization, a substantial portion of the Section Chief's time was taken up in residual work on NICHD scientific publications.

During the past year the Information Systems Section was engaged in making improvements to the existing PSAB computer programs and operational procedures.

One key change was the introduction of the IBM 2741 conversational terminal as the update mode for the Current Master File. Prior to this the operation was performed by DCRT personnel through a punched card routine. Delays were common, both in running the update routines and receipt of the material from DCRT. The new method enables PSAB to control the entire update process, including corrections and changes, and the time required has been reduced by about half.

In the same area of the computer activity of the Branch, another change was initiated which has made NICHD Master File more current. A schedule was established with the DCRT Input Operation to input our base data on a weekly basis. As a result of this, the Master File is within two weeks of being up-to-date with the receipt of the award statements in PSAB.

The Section also instituted an improved Master File input program for the scientific classification. This material now follows the same procedure as, and enjoys a status similar to, the administrative and fiscal data.

In conjunction with the Statistical Analysis Section, an attempt to put out the monthly active reports on a more timely basis has been undertaken. Currently being evaluated with Institute staff is an experimental issue based on an earlier cut-off date and processed through the Xerox Computer Forms Printer. If this method is adopted, the three to four week print shop delay could be avoided.

A substantial portion of the Section's activity was dedicated to the processing of the Federal Inventory for Population Research. The automation of this publication exacted a major portion of the time of the Section's staff, and will pay off in the future by enabling the Branch to handle later issues of this publication in a more efficient manner.

In another area, the Section is working with the Research Analysis Section to develop a more expeditious input mode for getting research summaries on the computer file. An experimental method being evaluated at present is the utilization of the Conversational Terminal and the DCRT WYLBUR Program.

A great deal of analysis and planning for future systems improvements is taking place within the Section. The Section will be setting forth a system design which will revamp and bring up-to-date the existing procedures.

Table 1. NICHD Grants and Contracts Active during March 1972
(including scientific evaluation grants)

Grants and Contracts Funded by NICHD

Mechanism of Support	Number						Funds (thousands)					
	Total	PR	PBIM	GD	ADA	MR	Total	PR	PBIM	GD	ADA	MR
Research Grants	833	275	192	215	55	96	$53,545	$14,270	$ 8,966	$12,465	$4,168	$13,676
Training Grants	138	30	27	38	25	17	11,132	2,500	2,165	2,767	2,271	1,330
Fellowships	64	12	19	19	8	6	667	109	199	195	101	63
RCP Awards	113	28	25	39	5	16	2,719	605	626	977	139	372
Total Grants	1,148	345	263	311	93	135	68,063	17,483	11,956	16,404	6,679	15,440
Contracts (by related program)	211	173	4	15	5	14	17,498	14,063	388	1,783	149	1,115
Total Grants and Contracts	1,359	518	267	326	98	149	85,561	31,546	12,345	18,187	6,828	16,555

Research Contracts by Organizational Unit

Organizational Unit	Number	Funds (thousands)
Total	211	$17,498
Center for Population Research	164	13,365
Fertility Regulating Methods Evaluation Branch	(15)	(3,788)
Behavioral Sciences Branch	(36)	(3,163)
Contraceptive Development Branch	(113)	(6,415)
Extramural Programs	27	3,103
Perinatal Biology & Infant Mortality Branch	(3)	(348)
Growth & Development Branch	(14)	(1,762)
Adult Development & Aging Branch	(4)	(113)
Mental Retardation Branch	(6)	(881)
Intramural Research Programs	13	518
Epidemiology & Biometry Branch	7	513

Notes: (1) Training grant totals include a scientific evaluation grant for $100,000 not assigned to a program.
(2) Dollar figures may not add to totals due to rounding.

NICHD - PSAB
March 21, 1972

Table 2. NICHD Grants and Contracts, Fiscal Years 1964-1971

Number

Mechanism of Support	1964	1965	1966	1967	1968	1969	1970	1971
Total Grants	1,013	1,222	1,444	1,531	1,393	1,331	1,176	1,147
Research	784	934	1,091	1,125	1,008	904	842	798
Training	74	92	132	146	150	151	134	127
Fellowships	88	119	141	166	135	173	86	100
RCP	67	77	80	94	100	103	114	122
Contracts	6	30	24	35	38	a/112	143	221

Funds (thousands)

Mechanism of Support	1964	1965	1966	1967	1968	1969	1970	1971
Total Grants	$27,017	$34,586	$45,451	$49,790	$51,396	$51,800	$53,106	$60,242
Research	21,612	26,524	34,303	36,787	37,985	37,596	40,099	46,314
Training	3,515	5,506	8,296	9,279	9,747	10,012	9,392	10,142
Fellowships	649	1,024	1,155	1,616	1,338	1,740	871	914
RCP	1,241	1,533	1,697	2,107	2,326	2,452	2,744	2,872
Contracts	138	1,264	946	2,520	2,150	a/6,310	7,678	16,122

a/ Includes 28 contracts totaling $1,496,000 funded through a PASA agreement with the Agency for International Development.

Note: Dollar figures may not add to totals due to rounding.

NICHD - PSAB
April 28, 1972

NICHD Annual Report
July 1, 1971 through June 30, 1972
Scientific Conference Branch

The responsibilities of the Scientific Conference Branch include the duties of Council Secretary, Committee Management and the management of NICHD operated conferences.

The National Child Health and Human Development Advisory Council met in November, March and June with the full complement of members. The Council was expanded to 14 members to provide additional coverage in population research and to permit the appointment of a youth member in accordance with Departmental recommendations.

The NICHD Committee Management Office has processed 34 appointments under the new guidelines for appointments during FY 72. There are currently 8 NICHD Advisory Committees including the National Advisory Child Health and Human Development Council. A total of 94 members are serving on these committees.

In compliance with the September 29, 1971, memorandum of the Secretary, HEW (with continuation March 29, 1972) instituting an Action Plan for Appointment of Women to Advisory Committees, this Institute has 12 women serving advisory committees as of March 31, 1972. It is anticipated that an additional 10 women will be appointed by the end of this calendar year. At this rate 23% of the membership of NICHD advisory committees will be women whereas the Departmental goal is one female for every two males.

In FY 72 this Branch assisted in the planning and arrangements for 17 conferences under the direct sponsorship of NICHD. Approximately 543 scientists participated in these meetings; less than half were first-time participants in Institute conferences.

The conferences by program area were:

Office of Program Planning and Evaluation

5th Conference on Behavioral Sciences and Medical Education

Center for Population Research

Workshop on Effects of Population on Human Behavior

Workshop on Developing and Education of Psychologists in the Population Area

Research Conference on Natural Family Planning

Perinatal Biology and Infant Mortality Branch

Intravenous Nutrition for High Risk Infants

Narcotics, Pregnant Women and Their Children

Amino Acid Requirements for Total Intravenous Nutrition in Infants

Growth and Development Branch

Workshop on Development of Motivation in Early Childhood

Workshop on Development of Motivation in Childhood

Structure and Biological Function of Immunoglobulin E

Adult Development and Aging Branch

Drug and Hyperbaric Oxygenation Therapies and Lucidity in the Aged

Characterization, Transfer and Maintenance of Aging and Defined Laboratory Rats

Cell Culture and Aging: Evaluation and Programming of ADAB Activities

Epidemiology of Aging

Mental Retardation Branch

Human Sexuality and the Mentally Retarded

Intramural Research Programs

Conference on the Pineal Gland

Symposium on Hormonal Control of Spermatogenesis

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Office of the Scientific Director

Intramural research programs have undergone expansion and reorganization during this fiscal year. The Developmental Immunology Branch has been established with Clinical Director, Dr. John Robbins, as Chief. The Pregnancy Research Branch has become operational under Dr. Ronald Chez, who has been engaged in planning a three-floor obstetrics and nursery unit to be added to the G wing of the Clinical Center in addition to conducting his own research program. The Section on Molecular Genetics of the Laboratory of Biomedical Sciences has been elevated to Branch status, with Dr. Phillip Leder as Chief. The Developmental Biology Branch has been abolished and its personnel and functions have been assigned to the new Laboratory of Molecular Genetics.

In fiscal year 1972 total budgeted positions for intramural research increased from 244 to 267. In addition a total of 85 scientists worked in NICHD laboratories as Guest Workers, Guest Scientists and Visiting Fellows. Another 34 employees in part time, temporary, WAE, stay-in-school, and work-study categories aided in our research programs. Renovation of laboratory facilities in Building 6 has been completed, and several of our programs have moved into this space. The improved facilities and capability to geographically unite related programs that had been dispersed will facilitate collaboration and scientific progress.

Developmental Immunology Branch - Accomplishments

1. The finding of bacterial antigens that cross-reacted with the capsular polysaccharide antigen of Hemophilus influenzae type b prompted a search for bacteria with cross-reacting antigens to Groups A & C N. meningitidis and Types I and III D. pneumoniae. Approximately 3,400 different bacteria of known serotype were studied. A number of bacteria were found with cross-reacting antigens to each. Infection of animals with these non pathogenic bacteria produced potent antibodies to the disease-producing organisms. These findings suggest both that infection by benign bacteria with cross-reacting antigens to pathogenic bacteria may be the source of "natural" immunity, and that artificial colonization with these bacteria may provide a new means of immunization. Evidence to support this hypothesis has been obtained. Two strains of E. coli were identified which contained a cross-reacting antigen to the capsular polysaccharide of Hemophilus influenzae type b. Newborn rabbits fed these strains of E. coli exhibit colonization, development of serum antibody, and apparent protection against a lethal challenge with H. influenzae, type b.

2. Human alpha 1-antitrypsin was isolated in a fully active form from whole serum. Immunologic testing showed that it was heterogeneous, consisting of two proteins of almost identical molecular size. Patients with homozygous deficiency of alpha 1-antitrypsin lacked the major component but retained the second. Thus an absence of only the major component is apparently related to the pulmonary emphysema seen in patients with alpha 1-antitrypsin deficiency.

Social and Behavioral Sciences Branch - Accomplishments

1. A time-sampling observational technique has been developed and refined for describing children's experiences in preschool settings. This instrument was used to evaluate the effects on children of different types of preschool programs. Children in a child-centered program (Bank Street model) showed significantly more fantasy play and self-expressive behavior, whereas children in a prepared environment program (Montessori model) showed a greater amount of time spent on pre-academic activities, such as reading, writing, quantitative skills and visual discrimination.

2. Another observational instrument has been developed and used to investigate the effects of early environmental influence on cognitive and personality development. Analyses indicate that kinesthetic stimulation (rocking or jiggling the baby or carrying him around the house) is especially important and is significantly related to the infant's mental and motor developmental status, social responsiveness, and cognitive motivational functions. The evaluation also documented a severe grossly depressing effect on function resulting from environments low in both social and inanimate stimulation, but did not find evidence that high social and inanimate stimulation could enhance vastly accelerated development.

Reproduction Research Branch - Accomplishments

1. Sensitive assays have for the first time been developed which specifically measure and discriminate between human chorionic gonadotropin (HCG) and luteinizing hormone (HLH), thus overcoming a major obstacle in reproduction research. These assays are so sensitive that scientists can now detect HCG as early as the 5th day after conception. Thus pregnancy is detectable 5 weeks earlier than previously possible. The demonstration that HCG is present this soon after conception challenges traditional concepts of reproductive biology, and suggests either that implantation occurs earlier than previously believed (7-9 days after conception) or that HCG is produced by the conceptus before implantation occurs. In addition, the assay has discerned ectopic production of an HCG-like hormone by a series of human neoplasms not previously known to have the capacity for HCG synthesis. The assay has proven uniquely useful for detection of these tumors and for following the response to treatment by women with trophoblastic neoplasms.

2. Hybrid somatic cell lines have been created by fusing choriocarcinoma cells with human and mouse fibroblasts. Several of the cell lines synthesized chorionic gonadotropin, showing for the first time that a specialized function can persist in hybrid somatic cells.

3. New radioimmunoassays have been developed for several steroids, including cortisol, dehydroepiandrosterone, androstenediol, 17-hydroxy-pregnenolone, and estriol. The resultant ease of analysis and improved sensitivity facilitated studies of adrenal and gonadal function. It was found that both the ovary and testis synthesize dehydroepiandrosterone, and that this steroid becomes a major secretory product during experimental stimulation. These assays also helped demonstrate for the first time that glucocorticoids can affect ovarian steroid secretion, a finding which necessitates reappraisal of

several functional tests of ovarian activity.

4. The first study of plasma concentrations of estradiol, estrone, estriol, and estrone sulfate throughout pregnancy has been completed. Estriol was present in lowest concentration. The ratio of estrone sulfate to the sum of estrone and estradiol was the same as in non-pregnant women. The quantitative significance, if any, of fetal estrone sulfate remains uncertain. Plasma concentrations of all steroids correlated with estimated fetal weight rather than with placental weight, emphasizing the known role of the fetus in maintaining maternal estrogen levels.

5. Studies in men seen in the male infertility clinic with a wide variety of testicular disorders support the hypothesis that tubular factors are important for FSH regulation. FSH levels are more generally elevated when tubular disease is more severe, but titers do not correlate precisely with sperm concentration. It is of interest that complete loss of germ cells is uniformly associated with elevated FSH, suggesting that one limb of a feedback loop has been interrupted and emphasizing the importance of the tubular epithelium in controlling FSH secretion.

6. A careful analysis of menstrual histories by scientists in the Reproduction Anthropometry Section has demonstrated a correlation between the length of the menstrual cycle and fertility. The conception rates were higher in women with longer cycles and in these same women the abortion rate was lower than in subjects with shorter cycles.

Behavioral Biology Branch - Accomplishments

Section on Brain and Behavior

1. Maternally-deprived rhesus monkeys have been studied as a model of the human maternal deprivation syndrome. In addition to the characteristic severe emotional and behavioral disturbance, the monkeys demonstrated EEG abnormalities. Chronic recordings from electrodes implanted in limbic structures demonstrate ongoing spike activity in the hippocampus, and suggest that these areas are functionally abnormal in this animal model of deprivation.

Section on Neurobiology

1. In order to study synthesis of various nerve cell constituents and their axoplasmic transport, reliable ultramicrotechniques have been developed both to introduce substances into single cells and to isolate any proteins synthesized following stimulus. A pacemaker neuron in a land snail has been found to synthesize a characteristic protein when active but not during hibernation. The same protein has also been isolated from active neurons of another species, Aplysia. These studies at the single-cell level are basic to understanding the mechanisms involved in mediation of neuronal function.

2. Hybrid lines of cells have been successfully formed between neuroblastoma cells and human diploid fibroblasts, and studied with regard to morphology, electrical excitability, and chemosensitivity. Some neuroblastoma cells exhibit differential chemosensitivity to acetylcholine. When this agent is

applied to the cell body it produces an excitatory effect, whereas application to the cell processes produces an inhibitory effect.

3. A procedure has been developed for selectively labelling cell membranes with ^{125}I in order to monitor procedures for membrane isolation. Separation and analysis of membranes is a crucial aspect of many neurobiologic studies, since such properties as electrical and chemical excitability and development of interneural communication are to a great extent a function of cell surface membranes.

4. Continued experiments with chick muscle and nerve cells grown together in tissue culture have demonstrated formation of neuromuscular junctions and neural synapses. Dorsal root ganglion cells grown in the presence of muscle cells are larger than if grown alone, suggesting a trophic interaction between these two cell types. The mechanism of this metabolic interaction is being investigated.

5. Studies of neuronal membrane permeability have demonstrated that salicylates and related nonnarcotic analgesics increase membrane permeability to potassium and decrease chloride permeability, and that there is a good correlation between their effectiveness in altering neuronal membrane permeability and their relative analgesic potency.

Laboratory of Molecular Genetics - Accomplishments

1. Research directed at understanding the processes involved in transmission of genetic information from one generation to another has enabled scientists in this laboratory to synthesize DNA complementary to the gene for rabbit globin. Purified rabbit globin mRNA was used as the template for transcription, mediated by avian myeloblastosis virus reverse transcriptase. This outstanding accomplishment represents the first step in synthesizing a complete mammalian gene.

2. Continued studies of the integration and excision of bacteriophage into and from the host chromosome have demonstrated that the process occurs with great precision at a number of sites such that normal function of the bacterium is restored. A variety of different transducing phages have been selected for the further study of the mechanism of genetic regulation and gene substitution.

3. Embryonic chick lens fibers are being used as a model system for studying cell differentiation. Elongation of the fibers at 15 days is associated with greatly increased RNA synthesis, and dependent on intact microtubules. It was found that insulin will substitute for serum in the culture medium of elongating cells, thus permitting for the first time the study of differentiation in a completely defined medium. This discovery has broad significance for tissue culture work in general.

Laboratory of Biomedical Sciences - Accomplishments

Section on Developmental Enzymology

1. Studies are continuing of placental and fetal enzymes and isozymes. Administration of steroid hormones to nonpregnant females was found not to increase serum hexosaminidase B, indicating that elevation of this serum enzyme in pregnancy is not necessarily mediated by steroid-producing organs such as the placenta or ovary. Electrophoretic analyses demonstrated that acid phosphatase in choriocarcinoma differs fundamentally from the enzyme in term placenta.

Section on Intermediary Metabolism

1. A laboratory animal model of mental retardation has been developed for the first time. Newborn rats treated with p-chlorophenylalanine and phenylalanine developed a biochemical picture similar to phenylketonuria. As adults these animals had behavioral alterations analagous to those observed in humans with phenylketonuria, including hyperactivity, deficiencies in problem solving, and smaller brains.

2. The synthesis of RNA in rat brain has been studied in a brain slice culture system. Synthesis of mRNA has been observed in this system, and attempts are being made to use it to prime the cell-free synthesis of brain-specific proteins.

Section on Physiologic Controls

1. A model system using fetal liver explants in culture has been used to study development of hormonal and nutrient control of enzymes involved in glycogen metabolism. A direct effect of insulin on net glycogen synthesis has been demonstrated for the first time using this system. This response increases rapidly on the first day after birth and is related to increased activation of the enzyme glycogen synthetase by a mechanism requiring protein synthesis, since it is prevented by agents preventing translational steps.

2. Studies of indole metabolism in the pineal gland have shown that serotonin and melatonin levels are controlled by the activity of the enzyme serotonin N-acetyltransferase. This enzyme is induced by a mechanism requiring cyclic-AMP and inactivated by photic stimulation. The neural pathway regulating N-acetyltransferase activity in the pineal gland has been shown to include the retino-hypothalamic projection and the suprachiasmatic nucleus. This nucleus appears to be the "biological clock" which drives circadian rhythms in the pineal enzyme. Several pineal peptides that inhibit or enhance reproductive function have been discovered and are being investigated.

Section on Developmental Pharmacology

1. Subcellular processes involved in the induction of pharmacologically active enzymes by drugs, polycyclic hydrocarbons, and insecticides have

been studied. It has been demonstrated that one such enzyme, aryl hydrocarbon hydroxylase (AHH), is inducible in the liver by phenobarbital (PB) and in many tissues by polycyclic hydrocarbons (PCHC). Administering PB or PCHC to the pregnant animal induces AHH activity transplacentally in fetal liver and other tissues. Cigarette smoking by pregnant women induces this enzyme activity in the placenta, an observation that may be related to the reduced size of infants born to cigarette smoking mothers. Working with inbred strains of mice in vivo and in tissue culture, it has been discovered that AHH induction in response to systemically administered PCHC follows a simple autosomal dominant pattern. This dominant expression is furthermore detectable transplacentally in utero and in fetal cell lines maintained in culture. This research provides a model system for study of enzymes and processes that may be inducible by drugs or environmental contaminants and that may be under genetic control as well, thus offering a powerful tool for exploring pharmacogenetics.

Section on Molecular Structure

1. The solid-phase technique of peptide synthesis in combination with countercurrent distribution has been used to synthesize membrane-active peptides of the gramicidin series. Gramicidin B with full biological activity was synthesized in 60 hours. Employing the same techniques gonadotropin releasing factor has also been synthesized.

2. Nisin and the gramicidins have been found to display effects on intrauterine development by either inducing fetal resorption or inhibiting implantation. The mechanism is unknown but may be related to inhibition of synthesis of chorionic gonadotropin, or to the disruption of lysosomes either in the genital tract or the placenta.

Pregnancy Research Branch - Accomplishments

1. A primate model of diabetic pregnancy has been devised and used to study carbohydrate metabolism. Maternal pancreatic beta-cells of Rhesus monkeys were destroyed by Streptozotocin pre-conceptually. Later, glucose solutions were introduced by catheter into the fetal stomach, and sequential blood samples were obtained from the maternal and fetal circulations. Orally administered glucose did not enhance plasma insulin concentrations in the normal or abnormal fetus, in contrast to the hyperinsulinemia exhibited by the abnormal fetus when glucose was administered intravenously. Fetal beta-cell hyperplasia was found in the diabetic model. This model has validated the theory of fetal hyperinsulinism in diabetic pregnancies.

2. Sequential samples of amniotic fluid obtained from baboon and rhesus pregnancies demonstrated changes in phospholipid/sphingomyelin ratios near term similar to those seen in human pregnancies. Changes in this ratio have been proposed as indicators of fetal maturity. Ligation of the trachea in these fetuses lead to nondistended lungs and oligohydramnios, whereas esophageal ligation resulted in polyhydramnios and fetal death. This model can be used to study possible ways of inducing fetal lung maturation.

3. A new method has been devised to destroy the fetal pituitary in utero

without interrupting the pregnancy. The method utilizes radiofrequency delivered through a probe inserted via the orbit into the sella turcica to induce hypophyseal lesions. This model will be used to study the effects of fetal hypophysectomy on length of gestation and adrenal-gonadal histology.

Developmental Biology Branch - Accomplishments

1. The personnel and functions of this Branch were transferred to the new Laboratory of Molecular Genetics during this fiscal year. Accomplishments are summarized under that laboratory.

Children's Diagnostic and Study Branch - Accomplishments

1. A clinical evaluation of hyperactive children on medication demonstrated marked effectiveness of dextroamphetamine and less effectiveness of chlorpromazine in reducing hyperactivity, compared with activity when the children received no drug or a placebo. Blood lactic acid levels and urinary catecholamine excretion were unrelated to the child's anxiety level as assessed by observers.

2. Patients with Down's Syndrome have low levels of platelet serotonin. Some investigators have suggested that the mental retardation of this syndrome may be due to abnormal CNS metabolism of this neurotransmitter. In order to investigate this hypothesis, levels of platelet serotonin were assessed in a number of patients with Down's Syndrome, who then underwent lumbar puncture to assess CNS metabolism of serotonin. The studies revealed that while platelet serotonin was 61% below normal in these patients, there was no abnormality detected in CNS serotonin metabolism or transport. Both patients and controls had a 3-fold rise in CNS 5-HIAA concentration (the principal serotonin metabolite) when probenecid was administered.

Gerontology Research Center - Accomplishments

Laboratory of Behavioral Sciences

1. A number of studies have focused on changes in learning that occur with age. In one experiment, young rats readily learned a difficult 14-unit T-maze, but old rats could not. The reason for failure by the old rats was shown to be that they made perseverative errors. Individual rats consistently exhibited an incorrect response at the same point in the maze, though the point varied from rat to rat. These older rats, however, could be successfully taught to complete the maze and correct their errors by concentrating their training into a short time span. By contrast, this type of training consistently led to poorer learning among younger animals. These findings indicate that the mechanism of learning may be different for the elderly than it is for the young, and that methods can be devised to produce effective training programs in the elderly.

2. Research has continued on learned control of cardiac arrhythmias. A patient with the Wolff-Parkinson-White syndrome was able to learn to control her cardiac function to such an extent that she could regulate not only her heart rate, but also to either produce or inhibit premature beats. This

finding suggests that rather exquisite extrinsic neural control of the heart is possible and learnable.

Clinical Physiology Branch

1. One of the best-documented physiologic changes that occurs with aging is alteration of carbohydrate metabolism. In order to explore this phenomenon a reproducible method was developed for controlling carbohydrate metabolism in human subjects sufficiently precise to permit controlled perturbation of the system as well as systematic alteration in the components. This technique has led to the demonstration that insulin is distributed in 3 body pools: Pool 1 corresponds to plasma volume, 4.6% of body weight; Pool 2 is small, lacks anatomic definition, is 1.9% of body weight, and in rapid equilibrium with Pool 1; Pool 3 represents interstitial fluid volume, is 9.3% of body weight, and is in slow equilibrium with Pool 1. Together these 3 pools represent the extracellular space. Measurement of insulin concentration in Pool 1 permits calculation of the hormone content in Pools 2 and 3. Delay in response of peripheral tissues to changes in plasma insulin concentration represents the time needed for insulin to diffuse from Pool 1 to Pool 3. Studies designed to compare young adults with aged subjects have demonstrated no difference in pool sizes or insulin kinetics, thus indicating that differences in carbohydrate metabolism between young and old subjects cannot be ascribed to hindrances in diffusion of insulin across the capillary barrier or through the interstitial spaces, as some theories of aging have suggested. An alternative hypothesis has emerged which is consistent with all available data: in the aged, insulin release from islet tissue shows an age dependent inertia.

2. Tests of renal function as determined by 24-hour creatinine clearance in volunteers in the Longitudinal Aging Study have indicated that loss of function accelerates as age advances. Young adults show a decline of only 2 ml per minute per decade, while aged adults lose 18 ml per decade. This finding contrasts with data from previous cross-sectional studies which indicated a linear decline of 9 ml per minute per decade over the adult age span, and points out the value of longitudinal studies.

3. The Longitudinal Study volunteers were also used to assess nutritional status in the elderly. Despite their general high educational level and upper-middle class status, 20% of the subjects not taking vitamin supplements had low levels of thiamine (B_1) and riboflavin (B_2), and 50% were deficient in pyridoxine (B_6). The group taking supplements had essentially no vitamin deficiencies. Attempts now must be made to determine if correlations exist between vitamin deficiency and rate of aging or aging-related diseases.

Laboratory of Cellular and Comparative Physiology

1. Studies of age-related changes in enzyme levels in the rotifer have shown that eight isozymes of acid phosphatase are present. Of these, five remain constant throughout the lifespan, one decreases, and two increase. The activity of the isozyme which changes most with aging shows a marked increase at the time of cessation of reproduction and a continuous increase

thereafter as high mortality rates appear. These observations support the hypothesis that aging may be a programmed phenomenon.

Laboratory of Molecular Aging

1. A chemical model has been developed to test the hypothesis that senescence results from errors in the incorporation of amino acids into proteins caused by mispairing of DNA or RNA nucleotide bases during replication, transcription, or translation. In the absence of divalent metal ions (Mn^{++}, Mg^{++}, Ni^{++}), it was found that poly (I) reacts only with the complementary cytidines in the copolymer (U, C), whereas if the metal ions are present, poly (I) reacts with both cytidines and uridines. The divalent metal ions thus can be used to induce mispairing in a consistent way, and this process and its effects can be studied.

2. New RNA-DNA hybridization conditions have been established wherein virtually all of a 28s ribosomal RNA is converted to a hybrid structure and is largely resistant to ribonuclease. A procedure for carrying out these RNA-DNA hybridizations with RNA covalently linked to filters has been successfully worked out. This system has potential application to the isolation of single genes in pure form and determining changes during senescence.

Collaborative Guest Scientist Program

In the past year 10 guest scientist programs were in progress at the GRC. Staff included 26 investigators and 32 support personnel. A number of important observations have come from this program.

1. Treatment of cells in tissue culture with hydrocortisone resulted in an increase of 30-40% in the number of cell divisions and an increased lifespan. Amounts of hydrocortisone below 5 μg/ml were ineffective, and 100 μg/ml had no more effect than 5 μg. Of other steroid hormones tested, cortisone and progesterone had some effect and others were ineffective.

2. Deposition of lipofuscin (age pigment) occurs in male accessory sex glands with aging. Systematic electron microscopic studies on rats have shown that the steps in the development of lipofuscin include segregation of organelles within membrane-bound spaces, followed by degradation and partial digestion of organelle components, and finally deposition of undigestible residues. Similar changes were observed after castration, but at an accelerated pace. These results suggest that lipofuscin deposition in target organs may result from a decline in androgen levels during aging. Since androgens control synthesis of structural proteins and building blocks to replace obsolete components of cytoplasmic organelles, any decline in androgen levels could lead to abnormal accumulation of undigested residues. This possibility is being investigated in long-term experiments in which rats are given different levels of exogenous testosterone.

Serial No. HD DB - 5

1. Developmental Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Immune mechanisms in chronic and congenital viral infections

Previous Serial Number: Same

Principle Investigator: Lon R. White, M.D.

Other Investigators: George Nemo, Ph.D., Epidemiology Branch, NINDS
Jacob A. Brody, M.D., Epidemiology Branch, NINDS
Harrie Anne Sutton, biologist

Cooperating Units: Epidemiology Branch, C&FR, NINDS
(corresponding project serial no. NDS(CF)-70 E 1833)

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: 1. To establish one or more models of chronic and/or congenital viral infection in experimental animals. 2. To define the development of cellular and humoral immunity to the same and to heterologous antigens.

Methods employed: Neonatal and pregnant mice are injected with either a reovirus type 1 or the minute virus of mice (MVM). The persistence of virus in animals is determined by isolation and fluorescent and immunofluorescent techniques. Serum levels of hemagglutination inhibition antibodies to both agents are followed. Lymphocyte transformation and cytotoxic activity is studied in spleen cell cultures in the presence of phytohemagglutinin on specific antigens. Any tumors which may develop are studied by light and electron microscopy. Extracts of MVM-containing tumor cells will be injected into newborn mice in addition to being studied in tissue culture.

Major findings: Attempts to infect embryonic animals by maternal infection in the first week of pregnancy have failed, presumably because infected embryos are reabsorbed. Late gestation maternal infection of 8 animals has resulted in the live birth of 53 animals, 39 of which survived to adult life. All progeny have appeared to be normal, most having been observed for 1 year. Two such animals have died of solid tumors; one of these was lost to study and the

other was found to have a tumor in the area of the right uterine horn with hepatic metastases. Extracts of both the tumor and liver yielded a hemagglutinating material presumed to be MVM antigen, suggesting that the animal was either reinfected late in life or had been chronically infected since fetal life. Cell-free materials from these tissues are now being studied both *in vivo* and *in vitro* in an attempt to define the possible role of MVM, either directly or indirectly, in the etiology of the tumor.

Significance to biomedical research and the program of the Institute: Infection of the human embryo or fetus with rubella or cytomegalovirus is associated not only with developmental abnormalities and mental retardation, but also with infection persisting for months or years after, despite the presence of neutralizing antibody in the serum. There are several examples of related phenomena in experimental animals infected during prenatal or neonatal life. Previous studies have suggested that an impairment of the immune function of lymphocytes may be associated with such persistent infection. These phenomena provide insight into the question of how viral infection is normally terminated, and represent a challenge to older ideas on the role of specific cellular immunity in the natural history of virus infections. The investigation described represents a direct experimental approach to elucidating the cause and course of chronic infection following initial exposure to the agent during immunologic immaturity. The results of this investigation will be of immediate relevance to our understanding of other types of illness known or suspected to be associated with chronic viral infection. In addition, they may suggest new approaches to research in the role of congenital viral infection as a cause of diseases of unknown etiology such as prematurity, idiopathic growth failure, malignancy, mental retardation developmental malformation, and autoimmune diseases.

Proposed course: To be continued.

Honors and Awards: None

Publications: None

Serial No. HB DB - 6
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Immune mechanisms in experimental allergic encephalomyelitis (EAE)

Previous Serial Number: Same

Principal Investigator: Lon R. White, M.D.

Other Investigators: George Nemo, Ph.D.
Marian Kies, Ph.D., Section on Myelin Chemistry, NIMH

Cooperating Unit: Section on Myelin Chemistry, Laboratory of Cerebral Metabolism, DBBR, NIMH
Epidemiology Branch, C&FR, NINDS (corresponding project serial no. NDS(CF)-70 E 1834)

Man Years:

Total:	0
Professional:	0
Other:	0

Project Description:

Objectives: To demonstrate in vitro "activation" of lymphocytes from guinea pigs with EAE by a myelin derived basic protein.

Methods employed: Techniques of lymphocyte cytotoxicity and blastogenic transformation have been employed.

Major findings: No cytotoxicity has been demonstrated. Transformation has been observed in some, but not all, experiments. These results seem to relate to the following variables: a) the "batch" of basic protein used, b) allotypic identity or dissimilarity between lymphocytes and target cells, c) strain of guinea pig utilized, d) interval between inoculation on animals and testing of lymph node lymphocytes.

Significance to biomedical research and the program of the Institute: EAE is generally held to be pathogenically similar to certain human demyelinating diseases, particularly the post-infections encephalitides and multiple sclerosis, and may involve lymphocyte-mediated autoimmunity. The in vitro demonstration of a basic protein "activatable" lymphocyte would be of great value to a more complete understanding of the experimental disease and would suggest new approach to the human diseases. The knowledge gained relating

to lymphocyte function would be of immediate relevance to problems of autoimmunity and chronic infection in certain diseases definitely or possibly due to viral infection during prenatal and neonatal life.

Proposed course: This project is terminated.

Honors and Awards: None

Publications: None

Serial No. HD DB - 7
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Specific Cellular Immunity in Recovery from Viral Infection

Previous Serial Number: Same

Principle Investigator: Lon R. White, M.D.

Other Investigator: Harrie Anne Sutton, biologist

Cooperating Unit: None

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: To determine the role and mechanism of acquired, specific, cell-mediated immune responses in recovery from viral infection *in vitro* and *in vivo*.

Methods employed: The initial studies have utilized infection of mice with MVM (minute virus of mice) and vaccinia virus. Spleen cells from control or immunized animals have been put into culture with cells previously infected with the appropriate agent. The following variables are: a) virus replication, b) virus-induced cell injury, c) cell-induced cell injury (lymphocyte cytotoxicity).

Major findings: No evidence of such immunity has yet been demonstrated.

Significance to biomedical research and the program of the Institute: It appears likely that specific cellular immunity may play a major role in termination of viral infections *in vivo* and in the host cell injury seen in certain viral infections. It is further hypothesized that an incompetence or abnormality of this mechanism may occur when the animal is first exposed to the virus during immunologic immaturity. The *in vitro* demonstration of such phenomena would represent a major advancement in our understanding of basic immune phenomena and the pathogenesis of virus-induced diseases.

Proposed course: To be continued.

Honors and Awards: None Publications: None

Serial No. HD DB - 15
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Search for a negative DNA strand in cells infected with the minute virus of mice (MVM)

Previous Serial Number: Same

Principle Investigator: Lon R. White, M.D.

Other Investigators: Harrie Anne Sutton, biologist
George Nemo, Ph.D., Epidemiology Branch, NINDS
Jacob A. Brody, M.D., Epidemiology Branch, NINDS
David Kohne, Ph.D.
University of California at San Diego

Cooperating Units: Epidemiology Branch, C&FR, NINDS (corresponding project serial no. NDS(CF)-71 E 1922)
Department of Microbiology, University of California at San Diego

Man Years:

Total:	0.4
Professional:	0.4
Other:	0.0

Project Description:

Objectives: a. To demonstrate a "template" DNA (negative strand) complementary to the single-stranded DNA (positive strand) of the MVM virion. b. To determine if the negative strand is associated with the cell's chromosomal DNA. c. To investigate the kinetics of negative strand synthesis relative to cellular DNA replication, cellular division, virus infection, and virus production. d. To utilize this method to detect a latent or cryptic viral infection in the cells of experimental animals and in tissue culture.

Methods employed: These studies utilize radioisotope "tagged" viral DNA. Annealing with "cold" DNA from virus infected cells is demonstrated by a) liquid scintillation detection of double and single stranded DNA in fractions eluted from a hydroxyapatite column, b) autoradiography with virus infected cells in metaphase. Some experiments use purified chromosomes and chromosomal DNA from mitotically phased cells in culture.

Major findings: Contrary to preexisting concepts, the DNA of the MVM virion appears not to exist as a simple, single "positive" strand: further

definition of the nature of MVM DNA will be required in order for the primary objectives to be accomplished.

Significance to biomedical research and the program of the Institute: Characterization of virus-cell interactions using a single-stranded DNA virus (parvovirus group, of which MVM is representative) is expected to advance our understanding of the mechanisms by which a virus infection might alter the genetic potential of the host cell (either a somatic or germ cell) as well as of mechanisms involved with the establishment of latent and chronic viral infections. Congenital, chronic, or latent virus infections may be involved with the etiologies of certain reproductive, developmental, and chronic diseases whose causes are now unknown. This experimental approach may prove to be directly applicable to a search for a parvovirus DNA in the cells in animals and persons with such diseases.

Proposed course: To be continued at an increased level of effort.

Honors and Awards: None

Publications: None

Serial No. HD DB -16
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Development and application of methods for the study of the minute virus of mice (MVM)

Previous Serial Number: None

Principal Investigator: Lon R. White, M.D.

Other Investigators: Harrie Anne Sutton, biologist
George Nemo, Ph.D., Epidemiology Branch, NINDS
David Kohne, Ph.D.
University of California at San Diego

Cooperating Units: Epidemiology Branch, C&FR, NINDS (corresponding project serial no. NDS(CF)-72 E 1980)
Department of Microbiology, University of California at San Diego

Man Years:

Total:	1.8
Professional:	1.0
Other:	0.8

Project Description:

Objectives: To develop improved methods for the propagation, purification, and quantitation of the virus and its nuclei acid. To investigate basic biological, biochemical, and structural properties of the virus and its DNA in order to develop techniques to investigate its persistence in congenitally and chronically infected experimental animals.

Methods employed: The agent is propagated in tissue culture and detected by cytopathic effect or antigen production. Its DNA is "tagged" during replication with tritium or P^{32}, or *in vitro* by iodination (I^{125}) on methylation (tritium or C^{14}). It is purified by differential centrifugation and by chemical means. The virus and its DNA are characterized by velocity sedimentation in sucrose gradients, electron micrographic appearance, salt and thermal elution characteristics from hydroxyapatite columns.

Major findings: Previous reports have described the growth of MVM in only two cell lines, both primary, with CPE in only one of these. We have extended this to 4 continuous cell lines, 2 of which show CPE. We have shown that whereas maximum virus production demands that the host cell divide, infection can occur in non-dividing cells and the virus can remain "latent"

without apparent injury to the cell until it divides. In addition, we have established chronic infection in two cell lines which have been producing virus in high concentration continuously for 16 months without apparent adverse effects on the host cell. We have accomplished a 100-1000 x improvement in yield of virus and hemagglutinating antigen and have shown that purification by isopycnic ultracentrifugation is associated with moderate virus disruption and loss of infectivity. Additional studies have revealed no significant loss of hemagglutination or infectivity titers with 3 cycles of freeze-thawing or with heating at 56°C for up to 90 minutes. UV inactivation progresses in approximately one $\log_{10}$ decrements per second under standard conditions. The in vitro infectivity titration method has been improved from a rather unreliable one-month procedure (which works poorly with "wild" strains of MVM) to a sharply reproducible, reliable one-week procedure usable with all strains. Methods are being developed to label viral DNA in vitro with H^3 or C^{14} dimethyl sulfate or I^{125}. In vivo labeling with H^3 thymidine or P^{32} had also yielded DNA of moderate to high specific activity. Preliminary results indicate that there is no DNA polymerase (DNA dependent) associated with purified MVM and that the virus may possess only a single protein. The nuclei acid, extracted by NaOH treatment and by a pronase-phenol-chloroform method, has been found to be extremely labile and "sticky," adsorbing to plastics, millipore filters, and dialysis tubing. Results of hydroxyapatite chromatography suggest that it may have a "snap back" area where it is double-stranded, that some virions may contain some "negative" strand DNA, and that the DNA may be packaged in segments.

Significance to biomedical research and the program of the Institute: MVM is representative of a group of small, single-stranded DNA viruses (the parvoviruses) which are characterized by ubiquity, generally low virulence and cytopathogenicity, an apparent preference for dividing cells, and a high frequency of transplacental infections. The study of this agent is expected to lead to a better understanding of mechanisms and the possible role of viruses in the causation of certain genetic. reproductive, and developmental abnormalities. MVM has been relatively unstudied, presumable because of technical problems. The improved methods which we have developed, and the basic information obtained by the application of these methods now make such studies feasible.

Proposed course: To be continued at present level of effort.

Honors and Awards: None

Publications: None

Serial No. LEP/NIAMD - 17
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on the relationship of chromosomal abnormalities and certain viral infections in mice. (Alternate title: Effects of viruses on mitosis and meiosis in the mouse.)

Previous Serial Number: Same

Principal Investigators: Beverly J. White, M.D., LEP, NIAMD
Lon R. White, M.D., DB, NICHD

Other Investigators: George Nemo, Ph.D., Epidemiology Branch, NINDS
Jacob A. Brody, M.D., Epidemiology Branch, NINDS
David Kohne, Ph.D., University of California at San Diego

Cooperating Units: Laboratory of Experimental Pathology, NIAMD
Epidemiology Branch, C&FR, NINDS (NDS(CF)-70 E 1839)
Department of Microbiology, University of California at San Diego

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: a. To localize within dividing mouse cells in vitro, particularly in relation to chromosomes, the site of residence of the infecting viral genome (of the minute virus of mice - MVM). b. To search for evidence of injury by virus to chromosomes of spermatogonia and somatic cells. c. To determine whether perinatal infection results in infection of the germ cells and, if so, to determine the duration of infection as well as the chromosomal and reproductive consequences of such infection.

Methods employed: The viruses under study are two strains of MVM and two strains of reovirus type 1. The animal used is the Cumberland View Farms C57BL/6 mouse. The viruses are "grown" and quantitated in tissue culture. Intracellular MVM genome (infectious or template DNA) is localized by hydroxyapatite column chromatographic demonstration of DNA - DNA hybridization using chromosomal DNA and radioisotope "tagged" MVM DNA. Chromosome studies are carried out on short-term spleen cell cultures as well as on cytologic preparations made directly from the spleen, testes, and bone marrow

of infected and control mice. Viral antigen is localized using immunofluorescent and immunohistochemical methods.

Major findings: a. A "fate" study in mouse spleen cells using tritium-labeled virus preparations showed no difference in autoradiographic grain localization between control and virus infected cultures. These were carried out with "tagged" viruses of relatively low infectivity and specific activity. b. Meiotic preparations from the testes of adult mice were studied 4 days, 1 week, 3, 6, and 12 weeks following MVM infection. The only notable observation was of a relative decrease in number of certain cell types, suggesting an effect on the course of spermiogenesis. c. Several animals initally infected in the perinatal period have produced normal litters.

Major improvements in methods involved with the propagation and quantitation of MVM have been accomplished. These have been used to produce viral "reagents" to be used in addition experiments. It was found that tritium labeling of viral DNA did not provide a sufficiently high specific activity to allow for autoradiographic localization of viral DNA in infected cells. An effort is being made to obtain high specific activity by labeling the DNA with I^{125} *in vitro*.

Significance to biomedical research and the program of the Institute: The role of viruses in the etiologies of chromosomal and developmental diseases is widely discussed, but negligibly investigated. The interaction of noncytolytic, non-oncogenic viruses with chromosomes and the spindle is also essentially undefined. The potential of these investigations is vast; basic information may be gained important to an understanding of human disease pathogenesis as well as to current concepts on the evolution of viruses and the basic nature of their interactions with dividing, differentiating cells.

Proposed course: To be continued at same level of effort.

Honors and Awards: None

Publications: None

Serial No. HD DB - 20(c)
1. Developmental Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on the consequences of viral infection in early life

Previous Serial Number: None

Principal Investigator: Lon R. White, M.D.

Other Investigators: Dwayne Reed, M.D., Epidemiology Branch, NINDS
Harrie Anne Sutton, biologist
Jacob A. Brody, M.D., Epidemiology Branch, NINDS

Cooperating Unit: Epidemiology Branch, C&FR, NINDS (corresponding project serial no. NDS(CF)-72 E 1979)

Man Years:

Total:	0.15
Professional:	0.1
Other:	0.05

Project Description:

Objectives: To investigate immune responses and neurologic development in children known or suspected of having contracted certain virus infections during infancy or early childhood. The initial studies have focused on infection with "natural" measles during the first year of life.

Methods employed: A population of approximately 100 children in and around Little Rock, Arkansas, known to have had measles before one year of age were identified with the assistance of a local hospital physician and public health officers. To test the feasibility of an intensive, longitudinal study of this cohort and control children, an attempt was made to contact, evaluate and obtain serum from several "case" and "control" children. Sera were tested for anti-measles antibody by hemagglutination inhibition.

Major findings: No "control" children could be identified. Five "cases" were examined and were considered neurologically normal although their antibody titers were slightly elevated above expected levels.

Significance to biomedical research and the program of the Institute: Arbovirus infections involving the CNS and occurring in infancy or early childhood are known to be associated with signs of minimal brain damage as the children near school age. This occurs even though the original illness was mild and followed by apparently complete recovery. Subclinical CNS involvement with the common childhood illnesses (measles, mumps, herpes

simplex, varicella, roseola infantum) is frequent; the neurologic sequelae of these illnesses are undefined but may be of considerable importance. This study represents an attempt to define the role of such early infections in the causation of minimal brain damage and related neurologic abnormalities.

Proposed course: It was decided that an extensive, longitudinal study of the Little Rock population would not be feasible.

Honors and Awards: None

Publications: None

Serial No. HD-DB-14

1. Developmental Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: RNA-instructed Nucleic Acid Polymerase Activity During Early Embryogenesis

Previous Serial Number: HD-DB-14

Principal Investigator: D. W. Slater

Other Investigators: None

Cooperating Units: None

Man Years

Total	: 1.0
Professional	: 0.5
Other	: 0.5

Project Description:

Objectives: To assess the role of RNA-instructed nucleic acid polymerases in early embryogenesis.

Methods Employed: Procedures have been developed for the preparation and assay of the pertinent polymerase activities as well as the resulting nucleic acid products.

Major Findings: Sea urchin eggs and embryos appear to contain at least two classes of holopolymerases capable of supporting DNA synthesis which are functionally distinguishable via their ability or inability to employ RNA as templates for such synthesis. Further, evidence has been obtained which suggests that the polymerases responsible for RNA-instructed DNA synthesis are themselves multiple in nature. Thus while RNA-directed DNA synthesis has been shown in all cases to be dependent on the addition of polymerase, template and appropriate cofactors and to result in an asymmetrically transcribed product the composition of which is dictated by the template employed, the differential kinetics of incorporation, divalent cation requirements, polymerase saturation levels, thermal labilities, as well as sensitivity to polymerase inhibitors, imply the existence of at least two discreet RNA-instructed DNA polymerase activities. We have also established that although the unfertilized egg is not engaged in DNA synthesis, it does possess a spectrum of similar, but not identical, latent polymerase potentials.

Hence, the complex of polymerases prerequisite to the DNA synthesis essential to early embryogenesis must, in the main, be synthesized during oogenesis.

Significance to Biomedical Research and the Program of the Institute: That all cell lines of a given organism are endowed with constant complements of nuclear DNA follows from the assumption that only DNA can beget DNA according to the semi-conservative dictates of specific base pairing. Nuclear transplantation experiments nothwithstanding, documentation of mechanisms which could support the systematic divergence of DNAs native to particular cell lines via amplification, magnification or promoter position effects, and do so within the confines of the central dogma, would necessitate a re-evaluation of the classical precept that differentiation is exclusively attributable to the emergence of divergent phenotypes from a constant genotype. Our results, which indicate that the embryo does contain DNA polymerases capable of employing RNA transcripts as templates for DNA synthesis, establish that at least one auxiliary mechanism is potentially operative during development which could effect such a divergence in the informational content of DNA. The identification of the mechanisms governing the expression of this elective during development is therefore deemed of primary importance to an understanding of the establishment of the differentiated state. That the potential to support the transfer of information from RNA to DNA appears to be an attribute common to both embryos and oncogenic viruses is also worthy of note.

Proposed Course of Project: This project will be continued with a major emphasis on the further purification of the relevant polymerases and their characterization during development. If logistically possible, it will be expanded to mammalian embryonic material.

Honors and Awards: None

Publications: Slater, Isabel, and Slater, D.W.: DNA Polymerase Potentials of Sea Urchin Embryos. Nature, in press.

Serial No. HD-DB-21

1. Developmental Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Utilization of Genetic Messages During Embryogenesis

Previous Serial Number: None

Principal Investigator: D. W. Slater

Other Investigators: D. H. Gillespie

Cooperating Units: Gerontology Research Center, NICHD, Baltimore, Maryland

Man Years

Total	: 1.0
Professional	: 0.5
Others	: 0.5

Project Description:

Objectives: To determine the mechanisms responsible for the processing and selective utilization of programs for protein synthesis which accompany early embryogenesis.

Methods Employed: Procedures prerequisite to the preparation and characterization of RNA from unfertilized sea urchin eggs and embryos associated with pertinent subcellular fractions have been developed which permit a direct assessment of the physical characteristics, synthesis and processing of poly A-containing genetic messages during early embryogenesis via molecular hybridization. The molecular hybridization procedures employed are novel in that they are based on RNA-RNA hybridization, rather than RNA-DNA hybridization. In essence, labeled poly U of high specific activity is hybridized to the unlabeled poly A sequences known to be covalently linked to genetic transcripts. The advantage of the RNA-RNA hybridization procedure employed over classical DNA-RNA hybridization methods which are dependent on in vivo labeled RNA and hence subject to ambiguities arising from fluctuations in endogenous precursor pools, permeability to labeled precursors, varying specific activities of the resulting RNA transcripts, difficulties in demonstrating that the labeled population on which the assay is based is indeed representative of the total existing spectrum of genetic transcripts, etc., is self evident. In addition, the procedure employed is also attractive in that workable specific activities of hybrids

can be assured without subjecting the cells in question to incubation in the presence of deleterious concentrations of labeled precursor or exposure to high energy labels such as P^{32} which frequently interfere with normal cell function. It should also be noted that the present procedure permits one to obtain meaningful results with nanogram quantities of unlabeled RNA.

Major Findings: The poly A content of sea urchin eggs and embryos has been examined with regard to the size of the poly A sequences per se, as well as that of the genetic messages with which they are associated during the course of early development. Our findings indicate that fertilization results in a rapid increase in poly A synthesis, as well as a transposition of pre-existing sequences. The size of the poly A sequences and that of the associated genetic messages have been found to be of surprisingly high molecular weight in comparison to that obtained from adult metazoans. However, as our investigations are the first to employ poly A as a naturally occurring marker to examine transitions in message distribution in embryos, we are presently uncertain as to whether this decided difference is attributable to the material employed or to the preparative procedures utilized. It is clear, however, that the patterns of genetic transcripts associated with poly A do undergo significant systematic alterations as a function of development. Manuscripts detailing the quantitative and qualitative transitions and transpositions of poly A and poly A-associated genetic messages during early embryogenesis are presently being completed for publication.

Significance to Biomedical Research and the Program of the Institute: The majority of metazoan genetic messages contain sequences of poly A. These sequences do not appear to be transcribed. While their actual function is presently unknown, the evidence available suggests that they are involved in the selective processing of RNA transcripts. In metazoans the synthesis of genetic messages is chronologically separable from their employment as templates for protein synthesis in terms of hours or even months. As it has been firmly established that embryogenesis in particular is contingent upon the ordered expression of such latent messages, it follows that mechanisms must be operative within the embryo which govern their selective and coordinate translation. The molecular basis of these regulatory mechanisms has yet to be determined. The apparent post-transcriptional association of poly A sequences with such messages has suggested that poly A is involved in the transport and utilization of genetic transcripts. The presence of these sequences, therefore, not only provides a unique natural marker with which to study the maturation and processing of genetic messages, but moreover offers an unparalleled opportunity to probe the mechanisms responsible for the selective utilization of programs for protein synthesis which occurs during early development.

As such information is a practical prerequisite to any attempts to exercise systematic remedial action on the course of development, this research carries obvious import both for our understanding of differentiation and for the therapeutic manipulation of embryogenesis. Further, as mentioned above, the poly A sequences present in the embryo are unusually large in comparison to those found in adult material. Indeed, the size of these sequences bears a striking resemblance to those known to be characteristic

of oncogenic viruses. It is therefore of interest to note that the embryonic material employed has also been shown by us to possess polymerases capable of supporting RNA-instructed DNA synthesis--the latter yet another attribute characteristic of oncogenic viruses. We are therefore not unaware of the additional possible implications of these findings for the etiology of carcinogenesis.

Proposed Course of Project: These investigations are being continued with the ultimate objective of elucidating the mechanisms responsible for the selective utilization of programs for protein synthesis during embryogenesis and their possible relationship to oncogenesis.

Honors and Awards: None

Publications: None

Serial No. HD-DB13
1. Developmental Biology Branch
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Ribosomal diversification during early development
I. The Non-coordinate synthesis of Ribosomal RNA and Ribosomal Protein
II. Recombination between maternal and embryonic subunits
III. Ribosomal-mediated recognition of specific genetic messages

Previous Serial Number: HD-DB13

Principal Investigator: D. W. Slater

Other Investigators: None

Cooperating Units: None

Man Years

Total: 0

Proposed course of project:

Research for this project has been temporarily suspended due to lack of personnel.

NICHD Annual Report
July 1, 1971 through June 30, 1972
Office of the Clinical Director

The research activities of the Office of the Clinical Director during this year were concerned with the scientific and applied problems concerning the nature, pathogenesis, and immunoprophylaxis of diseases in humans caused by Haemophilus influenzae type b. Research programs originally conceived in July 1970 to June 1971 were continued. Specifically, the research problems were concerned with the establishment of an animal model, the identification of the source of "natural" immunity, and characterization of the immune response of the capsular polysaccharide of Haemophilus influenzae type b when injected into children and infants.

Several research programs concerned with Haemophilus influenzae type b were undertaken. Specifically, genetic factors as measured by histocompatibility antigens and erythrocyte antigens were studied and related to the immune response and susceptibility to epiglottitis and meningitis. In addition, a survey conducted under a contractual mechanism at Charlotte Memorial Hospital was done to study the true frequency of this disease and its age incidence according to racial factors in the defined community. This survey was completed and revealed that if the data obtained from Mecklenburg County and Charlotte, North Carolina could be applied to the United States, then it would be expected that 12,000-15,000 cases would occur annually.

The Clinical Director's office was also concerned with establishing ties between the Society of Pediatric Research and the National Medical Research Council. In this light, the Clinical Director's office prepared an evaluation of phototherapy for bilirubin toxicity as treated by phototherapy in older children having gone through this treatment as well as to establish standards and references for manufacturers producing light therapy equipment. Such standardization has not occurred previously. In addition, the National Institute of Child Health and Human Development has undertaken a survey with several hospitals participating in the evaluation of phototherapy given to prematures by conducting neurological surveys on infants so treated.

Completion of a research program designed to devise new techniques for the isolation to permit more thorough characterization of the serum alpha-1 trypsin inhibitors of humans, and an experimental model of the mouse were completed. This research program resulted in two articles which were accepted for publication by Analytic Chemistry and Clinical Chemistry, and provide for the first time a reproducible method for the purification of the serum proteins. In addition, the proteins were found to be more heterogeneous than previously suspected and in both species, man and mouse, two serum alpha-1 trypsin inhibitors were discovered. This finding may permit a more precise analysis of the role of serum protein inhibitors of trypsin in the juvenile form of progressive emphysema.

The Office of the Clinical Director has continued its interest in understanding the nature prevalence epidemiology of Pneumocystis Carinii pneumonia. He has participated with the Center for Disease Control in evaluating the incidence of Pneumocystis Carinii pneumonia in infants and children with immunologic deficiency diseases.

Honors and Awards: None

Publications:

Robbins, J.B., and Western, K.A.: Pneumocystis Carinii Pneumonia. Brennemann's Practice of Pediatrics, Vol.II, Chapt. 52B, pp 1-8, 1972

Serial No. HD-TI(c)
1. Office of Clinical Director
2. Developmental Immunology Branch
3. Bethesda

PHS_NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on Human Serum Alpha-1 Antitrypsin

Previous Serial Number: None

Principle Investigator: Richard L. Myerowitz, M.D.

Other Investigators: Zeev T. Handzel, M.D.
John B. Robbins, M.D.

Cooperating Units: None

Project Description:

Objectives - The purpose of this study is to apply the principles learned in the investigation of mouse serum alpha 1-antitrypsin to the analogous human serum protein. The major objectives were to isolate the protein free from contaminants and still retain complete functional (anti-tryptic) activity. Also the second antigenic component seen in mouse serum was looked for in human serum and its relationship to the "slow component" of Laurell defined.

Methods Employed - For isolation of the protein the methods employed were DEAE cellulose chromatography, affinity chromatography with an insoluble immuno - (anti-albumin) adsorbent, and preparative polyacrylamide gel electrophoresis. Studies on the isolated protein utilized analytical polyacrylamide gel electrophoresis, isoelectric focusing, antigen-antibody crossed electrophoresis, anti-tryptic staining of gels, immunization of animals with analysis of antiserum by immunoelectrophoresis and immunodiffusion, and affinity chromatography with insolubilized trypsin.

Major Findings - Human serum alpha 1-antitrypsin could be isolated in a fully active form from whole serum by the methods described above. The isolated protein was markedly electrophoretically heterogeneous (as previously described by authors), consisting of a major component and five minor components, all of which were immunologically active against anti-alpha 1-antitrypsin serum and all of which were immunologically identical. This heterogenicity could be demonstrated in the alpha 1-antitrypsin of fresh whole serum.

The major component of alpha 1-antitrypsin was immunologically heterogeneous. Immunization of animals produced antiserum which detected two serum proteins in the isolated material and in fresh whole serum. Both proteins were antitrypsins and had almost identical molecular size and charge as determined by polyacrylamide gel electrophoresis. They were, however, immunologically distinct. One protein was present in much greater concentration and was called the major component. The other, newly described protein, was named the "second antigenic component" of alpha 1-antitrypsin. Patients with homozygous deficiency of alpha 1-antitrypsin lacked the major component except for the "slow component" of Laurell. However, they retained the second antigenic component which is, therefore, distinct from the slow component and presumably unrelated to the pulmonary emphysema seen in patients with homozygous alpha 1-antitrypsin deficiency.

Honors and Awards: None

Publications:

Myerowitz, R.L., Handzel, Z.T., Robbins, J.B., Human Serum Alpha 1-Antitrypsin; Isolation and Demonstration of Electrophoretic and Immunologic Heterogenicity, Clinical Chemica Acta, accepted for publication, in press.

Serial No. HD-CL-1(c)
1. Office of Clinical Director
2. Developmental Immunology Branch
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Identification and characterization of cross reacting antigens to the type b capsular polysaccharide of H. influenzae from mixed bacterial cultures.

Previous Serial Number: CL-1 (c)

Principle Investigators: Rachel Schneerson, M.D.
Zeev T. Handzel, M.D.
Richard Myerowitz, M.D.
John K. Whisnant, M.D.
John B. Robbins, M.D.

Man Years:

Total: 12/12
Professional: 12/12
Others: None

Project Description:

Objectives - To identify the prevalence in normal nasopharyngeal and stool samples, the presence of bacterial antigens cross reactive with the type b capsular polysaccharide of Haemophilus influenzae. In addition to this, on-going study techniques were devised to study bacterial cross reactions to the group A and C and meningitidis polysaccharides and to the capsular polysaccharides of D. pneumoniae types I and III. To identify the chemical structure, cellular localization, and immunogenicity of whole acetone killed and living bacterial vaccines in animals and to study the prevalence in nature of bacterial antigens cross reactive with the type b capsular polysaccharide of Haemophilus influenzae. In addition, the scope of the search for cross reactive antigens to capsular polysaccharide of pyogenic bacteria has been extended to the group A and C polysaccharides of N. meningitidis and the types I and type III capsular polysaccharides of D. pneumoniae. The work scope of this project was enlarged to include these four additional pyogenic bacteria because of several observations which had indicated that the wide prevalence of anti-capsular antibodies to the group A meningococcus could not be explained by a direct interaction with the homologous organism. Greater than 60 to 70% of children and young adults have anticapsular antibodies as detected by a variety of assays. Yet the group A meningococcus has not been detected in this country for the previous 20 years indicating that immunity to this group specific antigen was probably stimulated by an interaction with the cross reacting antigen.

5

Methods Employed - The low nasopharyngeal carriage rate of Haemophilus influenzae type b and the high incidence of detectable serum antibodies to this suggested that natural immunity was stimulated by bacteria other than Haemophilus influenzae type b. To test this hypothesis bacteria from mixed cultures of stool and the nasopharyngeal flora were studied for cross reacting antigens by using the anti serum agar technique with Haemophilus influenzae type b anti serum. A wide variety of bacteria were found to possess cross reacting antigens to Haemophilus influenzae type b. One bacterial antigen, a K or envelope antigen of Escherichia coli, was identified and the prevalence of this bacterium in stools of normal infants and infected fluids from individuals with E. coli infections was studied. It was found that approximately 3 to 7% of infants possessed this cross reacting bacterium in their stools and about 1% of urinary isolates taken from adult individuals possessed this envelope antigen. Among other bacteria, cross reactions to the Haemophilus influenzae type b polysaccharides were encountered in bacilli, lactobacilli, staphylococci, and streptococci. The common antigenic moiety of all these bacteria is polyribitol phosphate, usually present as a teichoic acid either in the cell membrane or in the cell wall. Currently the chemical basis for the serologic specificity of this cross reaction is under study.

Approximately three thousand bacteria previously studied for cross reactions to Haemophilus influenzae type b, were also studied for cross reactions to the group A and C meningococcus and pneumococcus types I and III. It was found that some Bacillis pumelis possessed a heretofore undescribed polysaccharide containing n-acetyl mannosamine phosphate, the specificity sugar of the group A meningococcus. Several E. coli possess an envelope antigen cross reactive with the group C meningococcus polysaccharide. Chemical analysis of these polysaccharides and inhibition reactions have confirmed specificity of these cross reactions. Cross reactions were encountered to the type I and type III pneumococcus among several E. coli. The identification of these cross reacting structures is still under study.

Injection of bacteria with cross reacting antigens to group A and C meningococci and types I and III pneumococci induced formation of serum precipitating antibodies toward the homologous structure in all instances. Thus, anti group A and C meningococcus polysaccharide sera and anti types I and III pneumococcal capsular polysaccharide sera could be raised by injecting the cross reacting antigens. Biological activity of these immune sera as well as the immunogenicity of the organisms fed to newborns is currently under investigation.

Honors and Awards: None

Publications:

Bradshaw, Major W., et al, Bacterial Antigens Cross Reactive with the Capsular Polysaccharide of Haemophilus influenzae type b. Lancet i:1095, 1971.

Schneerson, R., et al, An Escherichia Coli Antigen Cross Reactive with the Capsular Polysaccharide of H. influenzae type b, Journal of Immunology, in press.

Serial No. HO-CL-1(2)(c)
1. Office of Clinical Director
2. Developmental Immunology Branch
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30m 1972

Project Title: Identification and characterization of cross reacting antigens to the type b capsular polysaccharide of Haemophilus influenzae from mixed bacterial cultures and from serotypically defined Bacilli.

Previous Serial Number: Cl-1 (c)

Principle Investigators: Meir Argaman, Ph.D
Rachel Schneerson, M.D.
John B. Robbins, M.D.
Richard L. Myerowitz, M.D.
Zeev Handzel, M.D.

Other Investigators: J.C. Parke, Jr., M.D.
Cooperating Units: Charlotte Memorial Hospital, Charlotte, NC

Man years: 48/12

Project Description:

Objectives - To identify the chemical structure, cellular localization, immunogenicity in purified form and using whole acetone killed and living bacteria in animals, and occurence in nature of bacterial antigens cross reactive with the type b capsular polysaccharide of Haemophilus influenzae.

Methods Employed - Several experimental observations had directed our efforts to test the possibility that the "natural" immunity, observed in most adults and presumed to be due to serum anti capsular antibody, must be stimulated by bacteria other than H. influenzae. To test this hypothesis, a technique was used to identify bacteria with cross reacting antigens from mixed cultures taken from the gastrointestinal and pharyngeal flora of normal rabbits and humans. In this study, anti type b polysaccharide serum was incorporated into agar plates. H. influenzae type b and Diplococcus pneumoniae type 29 (an organism previously shown to contain a capsular polysaccharide cross reactive with the type b substance) yielded halos of precipitation surrounding the individual bacterial colonies, presumably due to the release of antigenically precipitable material into the antiserum agar. With this technique, an Escherichiae coli from a normal individual (designated strain Easter), several Staphylococcus aureus coagulase positive, S. epidermidis and several Streptococcus viridans were shown to yield a specific halo. To date, E. coli isolated from the collections at

the Center for Disease Control and W.H.O. have been compared to 25 E. coli isolated from routine cultures. Several serotypes with cross reactive polysaccharides have been identified. The material isolated from these E. coli has been shown to be capsular in cellular localization by immunofluorescence and to be a polysaccharide containing ribose and ribitol by direct analysis of the sugar. Isolation of the cross reacting material from all the cross reactive organisms has been accomplished but the high degree of purity achieved for the E. coli material has not yet been recognized. Immunization with whole encapsulated E. coli S. aureus, and Bacillus subtilis has produced antiserum shown to be protective in mouse lethal infection assays (using mucin enhancement) and in the complement dependent bactericidal assays with virulent stains of H. influenzae. Absorbtion of anti H. influenzae type b serum and anti E. coli serum with the cross reactive antigens has been shown to remove protective activity from both reagents. Although studies are imcomplete, preliminary evidence indicates that the antigen isolated from the three E. coli is protective, anti- genically identical to the polysaccharide of H. influenzae and occupies the same cellular localization. Its preliminary characterization would seem to put it in the classification of "K" antigen, usually acidic polysaccharides which are capsular. Using the 90 antisera present in the W.H.O. Escherichiae Center, no specificity of K has been attached to this material as of yet.

IIdentification of ribitol in the type b capsular polysaccharide of H. influenzae directed a search for polysaccharides containing this polyol. The teichoic acids of gram positive bacteria that are located within the cell wall of Bacillus subtilis, Stretococcus faecium (Group D), Lactobacillus plantarum, and Staphylococcus aureus, coagulase positive, were isolated and found to cross react with the type be capsular polysaccharide. Absorbtion studies and inhibition reactions with monosaccharides (ribitol phosphate, ribose phosphate, etc.) have indicated that the ribitol identified in the type b polysaccharide is antigenic and probably accounts for the cross reaction.

Honors and Awards: None

Publications:

Schneerson, R., Bradshaw, M.W., Whisnant, J.K., Myerowitz, R.L., Parke, J.C., Robbins, J.B., An Escherichia coli Antigen Cross Reactive with the Haemophilus influenzae type b Capsular Polysaccharide: Occurence Among Known Serotypes and Immunochemical and Biological Properties of E. coli Antisera toward H. influenzae type b, Journal of Immunology, (accepted for publication - June, 1972).

Serial No. HDI4(c)
1. Office of Clinical Director
2. Developmental Immunology Branch
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Identification of Non-Pathogenic Bacterial Antigens Cross Reactive with Meningococcal and Pneumococcal Capsular and Non-Capsular Structures.

Principal Investigator: John B. Robbins, M.D.

Other Investigators: Richard L. Myerowitz, M.D.
Zeev T. Hanzel, M.D.
John K. Whisnant, M.D.
Rachel Schneerson, M.D.
Meir Argaman, Ph.D
Emil Gotschlich, M.D.

Cooperating Units: Rockefeller University, New York City

Man Years:
Total: 42/12
Professional: 42/12
Other: None

Project Description:

"Natural" serum antibodies to the capsular polysaccharide of Neiserria meningitidis Group A have been detected in approximately 50 to 70% of children and army recruits despite the scarcity of this organism in the United States for the past 20 years. These "natural" antibodies have been directly related to immunity to disease with this organism. Previous identification of cross reacting antigens among non-pathogenic enteric bacteria to the capsular polysaccharide of Haemophilus influenzae type b prompted a search for cross reacting antigens to N. meningitidis Group A and C and D. pneumoniae types I and III. Approximately 3,400 bacteria from serotypically defined collections of the Center for Disease Control, Atlanta Georgia, and the W.H.O. Enteric Laboratory, Denmark, and enteric bacteria isolated from urinary tract infections, stool culture from infants with enteropathic diarrhea, blood, CSF and normal stool were studied for cross reactions by immunodiffusion analysis. Cross reactions to capsular and non- capsular structures of the four pathogens were detected among the enteric bacteria. The enteric antigens were isolated from fluid culture by fractional precipitation with hexadecyltrimethyl ammonium bromide, and phenol extraction.

N. meningitidis Group A:

Bacillus pumilis was found to contain a polysaccharide which showed a partial identity reaction with the group A meningococcal polysaccharide. The specificity of the reaction was confirmed by inhibition reactions with the nonosaccharide inhibitor N-acetyl mannosamine phosphate. Immunization with the cross reacting Bacillus pumilis resulted in potent anti Group A meningococcal serum. A survey of Bacilli from the collection of Dr. Ruth Gordon, Rutgers University, revealed that most B. pumilis have this antigen. Partial characterization of the cross reacting structure indicates that it has glycerol phosphate teichoic acid backbone with mannosamine phosphate side chains.

N. meningitidis Group C:

Several E. coli cross reacted with group C meningococcal polysaccha- ride. Analysis revealed Neuramanic acid in the E. coli polysaccharide. Immunization with the E. coli produced potent group C meningococcal anti-serum.

D. pneumoniae types I and III.

Several E. coli possessed cross reacting polysaccharides to the capsular polysaccharides of these two types of D. pneumoniae. Immunization with these E. coli produced potent pneumococcal type I and III capsular polysaccharide antibodies.

These studies indicate that cross reacting antigens to capsular polysaccharide antigens of pyogenic bacteria are demonstrable among non-pathogenic enteric organisms. The immunogenicity of these bacteria within the gastrointestinal tract, their potential for immunogens in humans, and their role and inducers of "natural" antibodies is under investigation.

Non-capsular cross reactions were observed for all four pathogenic bacteria among the same collection of enteric bacteria. Characterization of these structures has not commenced.

Honors and Awards: None

Publications:

Robbins, J.B., Myerowitz, R.L., Whisnant, J.K., Argaman, M., Schneerson, R., Handzel, Z., and Gotschlich, E., Enteric Bacterial Antigens Cross Reactive with Neisseria meningitidis and Group A and C Diplococcus pneumoniae types I and III, Journal of Infectious Diseases, accepted for publication.

Serial No. HD-CL-4
1. Office of Clinical Director
2. Developmental Immunology Branch
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Age Related Susceptibility to Haemophilus influenzae Type b Infections in Rabbits

Previous Serial Number: CL-4

Principal Investigator: Rachel Schneerson, M.D.

Other Investigators: John B. Robbins, M.D.

Cooperating Units: None

Man Years:

Total: 12/12
Professional: 12/12
Other: 12/12

Project Description:

Objectives - To determine if the inverse relation between serum capsular antibodies and susceptibility to H. influenzae type b meningitis shown to occur in humans can be demonstrated also for laboratory animals. It has been stated that H. influenzae type b is not a true pathogen for laboratory animals (based upon many observations which indicated that since large numbers of organisms must be injected with non-specific irritants to induce H. influenzae type b meningitis).

Method Employed - Serum anti capsular antibodies were measured in animal sera by use of a passive hemagglutination test. The organism employed to test the susceptibility of these rabbits to infection was H. influenzae type b, strain "Rab", utilized for the purification of type b polysaccharide.

The anti type b antibody was measured in the sera of rabbits of varying ages. Animlas were either taken from outdoor hutcheries of commercial breeders or the NIH.

Major Findings - The presence of anti type b antibodies in rabbits was similar to that observed in humans. Newborns and adult animals (greater than 6 weeks of age) had anti type b antibodies in their sera (approximately 95%). Animals from 1-3 weeks of age had no detectable type b antibody. Examination of the nasopharynx and rectum of animals of varying ages showed a variety of bacteria with cross reacting antigens to

the type b capsular polysaccharide, but in no case was H. influenzae type b isolated. We concluded that the antigenic stimulus for the synthesis of anti type b antibodies is probably other than H. influenzae type b.

Animals with pre-existing antibodies of about 1-3 weeks of age were highly susceptible to fatal septicemia with H. influenzae type b. Approximately 50% of animals without pre-existing anti type b antibodies died within a variable period of time (1 day - 2 weeks) following intravenous injection of 1 x 103 organisms. The mortality of animals injected with 105 bacteria was greater than 90%. Of interest was the observation that H. influenzae type b could be cultured in the blood for two weeks without any detectable change in the animals' general health and well being. Meningitis or pneumonitis was detected in many animals. Animals with pre-existing antibody, whether maternally acquired after birth or naturally acquired during adult life or administered passively, were highly resistant to levels of organisms which ranged from 105 to 107. At doses of 108 and 109 organisms all animals died soon after IV injection (less than 2-3 hours) due to toxic effects.

It was concluded that an experimental model for septicemia and meningitis with H. influenzae type b could be established in laboratory animals. The failure of previous investigators to establish a laboratory model for H. influenzae type b disease could be attributed to the presence of serum type b antibodies in rabbit sera following 8 weeks of age. The source of antigenic stimuli to produce anti type b antibodies is probably due to cross reacting antigens present on bacteria other than H. influenzae type b.

Honors and Awards: None

Publications:

Schneerson, R., and Robbins, J.B., Age-Related Susceptibility to Haemophilus influenzae Type b Disease in Rabbits, Infection and Immunity, 4:397-401, 1971.

Serial No. HD-CL-2
1. Office of Clinical Director
2. Developmental Immunology Branch
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Purification and Physicochemical Characterization and Biological Properties of Mouse Serum Alpha-1 Antitrypsin.

Previous Serial Number: CL-2

Principle Investigator: Richard L, Myerowitz, M.D.

Other Investigators: John B. Robbins, M.D.
Andreas Chrambach, M.D.
David Rodbard, M.D.

Cooperating Units: Reproduction Research Branch, NICHD

Man Years:

Total: 12/12
Professional: 12/12
Others: None

Project Description:

Objectives - A disease state has been described in humans characterized by a genetically controlled deficiency of a serum protein (alpha-1 antitrypsin) and a progressive, fatal, youthful emphysema unrelated to environmental exposure. The pathogenesis of this pulmonary lesion is unknown but the direct relation between the homozygous serum protein deficiency and the pulmonary affliction is well documented. The human serum protein exhibited microheterogeneity with an antigenically identical "fast" and "slow" variant in normals. The homozygous deficient state is characterized by an absolute deficienty of the"fast" variant.

The objectives of this study are to isolate the serum alpha-1 trypsin inhibitor in a laboratory animal (mouse), to identify the microheterogeneity and define any genetic polymorphism, to study by physiologic and biochemical techniques the possible physiologic function of this protein to, identify the biologic function, and to study the cellular basis of its synthesis (cellular localization and biosynthetic control.)

Serum alpha-1 antitrypsin has physical and chemical properties very similar to serum albumin. In man, purification of the functionally active serum protein has been difficult. In the mouse preliminary observations revealed that the electrophoretic mobility of the alpha-1 antitrypsin with respect

to serum albumin was dissimilar in two types of supporting gels. In polyacrylamide gels of high concentration it is observed that the electrophoretic mobility of this protein increased relative to albumin indicating that there was a molecular size and charge difference between alpha-1 antitrypsin and its nearest neighbor and usual contaminant, serum albumin. Accordingly, electrophoretic mobility of serum albumin and serum alpha-1 antitrypsin were determined in polyacrylamide gels of varying concentration and pH. By extrapolating these values, it was found that conditions could be achieved for preparative polyacrylamide gel electrophoresis for the separation of this alpha-1 antitrypsin from all other serum proteins. Preliminary to the preparative polyacrylamide gel electrophoresis procedure, serum albumin was removed from fractions separated by DEAE cellulose chromatogrpahy by passage through an insoluble anti-mouse column (affinity chromatography). The resultant preparation was found to be essentially pure and free of contamination by any other serum protein. The specific antitryptic activity, molecular weight, and carbohydrate content is now under investigation.

The biologic properties of this serum protein can now be investigated through studies of the action and metabolism of the purified protein in vivo using antisera raised against the purified protein.

To date, a molecular weight determination of 58,000 was done by the sedimentation equilibrium method. Antigenic analysis of this purified protein has revealed microheterogenieity with a "fast" and "slow" component, similar to those observed in humans. Unlike the heterologous antiserum, human alpha-1 antiserum system, antigenic differences are demonstrable between these variants. This antigenic difference, revealed by the antiserum, will be used to determine the cellular basis of the synthesis of these two variants.

Honors and Awards: None

Publications:

Myerowitz, R., Chrambach, A., Rodbard, D., Robbins, J.B., The Isolation and Physico-Chemical Properties of Mouse Serum Alpha-1 Antitrypsins, Analytical Biochemistry, May, 1972.

Serial No. HD-CL-6(c)
1. Office of Clinical Director
2. Developmental Immunology Branch
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Identification of human lymphocytes structures related to susceptibility to disease and related to the immune response to Haemophilus influenzae type b.

Previous Serial Number: CL-6(c)

Principal Investigator: John K. Whisnant, M.D.

Other Investigators: Dean Mann, M.D.
Nicholas Rogentine, M.D.
John Robbins, M.D.

Man Years:

Total: 21/12
Professional: 12/12
Other: None

Project Description:

This project is an extension of our previous attempts to delineate a human model for susceptibility to the infectious agent Haemophilus influenzae type b. Three objectives have been sought: (1) to expand our analysis of genetic traits in persons with two different H. influenzae type b diseases, meningitis and epiglottitis; (2) to prepare serologic reagents for testing lymphocytotoxicity as a marker for disease; and (3) to relate genetic traits studied to the immune responsiveness in the two groups of patients.

Segregating antigenic markers on erythrocytes and peripheral blood lymphocytes have now been studied in 50 patients and 130 of their first degree relatives. The distribution of red cell Rh and MNS antigens is different from a nondiseased population. These two patient groups also have different distributions of Histocompatibility antigens (HL-A). The mechanistic relationships of the HL-A antigen A11, increased in meningitis, or the red cell gene NS, increased in epiglottitis, to pathogenesis of disease are under evaluation.

Previous experiments have shown that human lymphocytes are killed in the presence of complement and purified anti H. influenzae type b antibody. The specificity for this cytotoxicity, as determined by dye exclusion, and its increased frequency in patients with epiglottitis is being pursued.

These cytotoxic antibodies are present in a small percent of nonimmune sheep as well as in hyperimmune sheep having received H. influenzae type b as opposed to types a or c organisms. Another variable in the cytotoxicity typing of human lymphocytes is the low level toxicity of fresh rabbit complement. Experiments are in progress to relate this "natural" cytotoxin to the level of H. influenzae type b antibody known to be present in adult rabbit sera.

The genetic disparity between meningitis and epiglottitis patients and the disparity in antibody levels between these patients suggests a model for study of genetic control of immune response in humans. The genetic traits determined in these patients seem, on preliminary data analysis, to be related to the individuals ability to form high levels of specific anti H. influenzae type b antibody. In particular, 5 of 7 children with very high antibody levels are of a single, rare histocompatibility antigen type.

Honors and Awards: None

Publications:

Whisnant, J.L., Mann, D., Rogentine, N., and Robbins, J.B.,: Human Cell Surface Antigens Related to Disease Cause by Haemohilus influenzae type b. The Lancet ii:895, 1971

Serial No. HD-I 8
1. Office of Clinical Director
2. Developmental Immunology Branch
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Identification of the immune response to Haemophilus influenzae type b capsular polysaccharide induced by neonatal feeding of bacteria with cross reacting antigens.

Previous Serial Number: None

Principal Investigators: Zeev Handzel, M.D.
Richard Myerowitz, M.D.
Rachel Schneerson, M.D.
John B. Robbins, M.D.

Other Investigators: None

Man Years:

Total: 12/12
Professional: 12/12
Others: None

Project Description:

Objectives - To identify the effect upon "natural" immunity to Haemophilus influenzae type b by feeding neonatal rabbits with bacteria containing cross reacting antigens to the capsular polysaccharide of Haemophilus influenzae type b.

Methods Employed - Neonatal animals have been fed two E. coli strains designated 075:H5 which contain a newly described cross reacting antigen to the type b polysaccharide. Colonization with these organisms was determined by culturing the stool and nasopharyngeal area with anti serum agar plates. Measurement of serum antibodies to the type b capsular polysaccharide as well as to E. coli antigens was done by radio immunoassay and a passive hemagglutenation test.

Significance of Work - Induction of "natural" immunity to Haemophilus influenzae type b by neonatal feeding of a cross reacting, non-pathogenic bacteria, might provide an alternate method for immunization against diseases caused by this organism. In addition, since analogs to the capsular polysaccharides toward other pyogenic bacteria including meningococci and pneumococci have been found, then, this method, if proved to be safe and immunogenic, might provide a general method for the artificial

and accelerated induction of "natural" immunity toward bacteria that cause meningitis.

Honors and Awards: None

Publications: None

NICHD Annual Report
July 1, 1971 through June 30, 1972
Office of the Clinical Director
Contract and Collaborative Research

Contract Title: Development and Testing of Capsular Polysaccharide of Haemophilus influenzae type b

Contractor: Charlotte Memorial Hospital
P.O. Box 2554
Charlotte,North Carolina

Money Allocated: $38,000

Objectives - To confirm the immunogenicity of the type b polysaccharide in infants and children. To study the optimum dose (serum antibody response) in infants and children. To determine the relationship between maternal serum antibody and the immune response to the type b capsular polysaccharide. To determine the effect upon the immune response to the type b capsular polysaccharide of the presence of bacterial antigens with cross reactive moieties to the type b capsular polysaccharide. To determine the overall incidence, the age distribution, and other factors such as economic and racial makeup of infants who have contracted Haemophilus influenzae type b diseases in Mecklenburg County.

Methods - To determine the optimum immunogenic dose of type b polysaccharide for infants as well as to study the effect of the immune response of the type b polysaccharide conferred by bacterial antigens with cross reacting antigens to the Haemophilus influenzae type b polysaccharide, all infants who leave the newborn nursery at Charlotte Memorial Hospital will have their stool cultures examined for cross reacting antigens by the anti serum agar technique and their level of anti type b antibodies as studied in the cord serum and maternal serum will be measured by the radioassay. When these infants return to Charlotte Memorial Hospital to participate in their continuing care program, the parents of two to three month old infants appearing for their first DPT injection will be interviewed and requested to have their infants participate in the immunization program. Blood samples, nasopharyngeal cultures, and rectal swabs will be obtained at one, two, three and six months following injection of the polysaccharide. Untoward reactions following the injection are to be studied by phoning the parents of the child within 24 hours following injection.

A survey of the prevalence of cross reacting antigens and the effect of non-typeable Haemophilus influenzae upon the immune response to the type b polysaccharide is also being studied. Four-day old infants with cross reacting bacterial antigens isolated from their stool will be studied for the sequential appearance of anti type b antibodies "natural" immunity, as compared to controlled infants and children without demonstrable cross reacting bacteria.

Honors and Awards: None

Publications:

Parke, J.C., Jr., Schneerson, R., Robbins, J.B.: The incidence, age and racial incidence, and mortality of Haemophilus influenzae type b diseases in Mecklenburg County from 1966-1970, J. of Pediatrics, accepted for publication in August 1972.

NICHD Annual Report
July 1, 1971 through June 30, 1972
Social and Behavioral Sciences Branch

Summary of Research Activities

This year the Social and Behavioral Sciences Branch has extended its research on early environmental influences to include studies from a new perspective. A study has been initiated to investigate the interaction between infant characteristics and maternal behavior. Until recently most research on the effects of early experiences has conceptualized the problem in terms of a simple antecedent-consequent model, attempting to assess the impact on the child of the environment. Rather than viewing the infant simply as a passive recipient of stimulation, in this new study a basic assumption is that the infant actively interacts with his environment, eliciting behaviors from his mother and others, and through his individual sensitivities selectively filters and moderates environmental events. In the current study we are attempting to elucidate the relationships between some specific characteristics of the infant and specific behaviors of the mother.

The study of the effects of infant temperamental characteristics on maternal behavior patterns builds on a methodological investigation of infant temperament carried out last year. The current investigation begins with interviews of the mother and father around the end of the prenatal period. It includes neonatal and four week assessments of infant characteristics, mother-infant observations in the home and interviews with the mother and father when the infant is four weeks old. In addition to the core problem of how specific characteristics of the infant, e.g., his responsiveness to social and inanimate stimuli, the ease with which he can be consoled, affect his mother's behavior towards him, several other basic questions will be studied: the relationship between the woman's physical and psychological adaptation during pregnancy and her adjustment to the maternal role; the question of whether there is a basic consistency in selected infant characteristics between the neonatal period and four weeks.

Our interest in the early environment has been extended to include observations of father-infant interaction. Most of our knowledge of early parental behavior is limited to the mother-infant unit. The apparent assumption that this is a closed system, relatively unaffected by the father, and that early paternal behavior is of no consequence is certainly open to question. Our study involves interviewing the father when the infant is four weeks old regarding his first contacts with the baby, his current relationship with him, and aspects of the husband-wife relationship which may bear on how each parent relates to the baby. At age five weeks an observation is conducted in the home, usually in the evening, focusing on the father's interaction with the baby. This study should yield useful descriptive data in its own right as well as increase our understanding of early family interaction.

During this year, work has proceeded on data reduction and analyses of data from two studies completed in the previous year. Several papers were presented at professional meetings discussing these findings.

In the investigation of early environmental influences on cognitive and personality development during the first half year, we have completed additional analyses. We have looked at the role of stimulation in specific sensory modalities in early development. The mother's activities with six-month old infants were analyzed in terms of visual, auditory, tactile and kinesthetic components. We find that kinesthetic stimulation is an especially important sensory modality. There was a positive and significant relationship between amount of kinesthetic stimulation and both the Mental and Motor Developmental Indices of the Bayley Infant Scale. This variable was also significantly related to measures of social responsiveness, goal directedness, object permanence and secondary circular reactions. Separate analyses for boys and girls revealed striking sex differences; these relationships were statistically significant only for girls. We cannot, however, simply explain these findings in terms of biologically based differences in sensitivity to kinesthetic stimulation. In looking at the data more closely we found that boys tend to receive higher levels of kinesthetic stimulation than girls. We also found that for the girls, the significant relationships are most clear in the lower range of stimulation. There seems to be a point beyond which increased amounts of kinesthetic stimulation have no appreciable effects on the developmental functions we measured. Thus there seems to be a complex interaction between sex of child, amount of kinesthetic stimulation received and its relationship to developmental functions. Dr. Frank Pedersen discussed these findings at the Southeastern Conference on Child Development in Williamsburg, Virginia.

An intensive followup study of this sample at three years of age was planned to study the interaction of early experiences and subsequent experiences in relation to personality and cognitive development. It was not possible to pursue this study because of lack of staff. However, a more limited study was carried out on these subjects--the collection of intelligence test data at three years of age. These data will allow us to look at changes in intellectual functioning during the first three years and to study the relationship between environmental variables in infancy and IQ at three years.

As part of our interest in early environmental influences, we have carried out further analyses of data obtained in a study of the impact of experiences in Headstart and day care programs of four to five year old children from disadvantaged backgrounds. Consistent with the findings of several studies of compensatory preschool programs, we found few significant relationships between specific cognitive experiences and changes in these cognitive functions. Cognitive activities tended to be very infrequent; other dimensions of teacher behavior, such as individualization of the children, giving positive feedback, also tended to be rare in these schools. The lack of significant changes in functioning might be partly related to the low level of cognitive stimulation and affective interaction; it might be related to

the relatively short time interval between pre and post tests--approximately six months; it might be a function of the insensitivity of the tests we used. It might also mean that intervention in one setting--the school--is not sufficient to offset the cumulative effects of disadvantaged environments. More focused work with the parents may be helpful to reinforce the positive learning experiences in the school.

In order to evaluate the effectiveness of intervention programs it is necessary to analyze in a very differentiated way the components of compensatory education programs. The observation technique which we developed for the study of Headstart schools seemed to be a promising tool for the study of preschool environments. Therefore we instituted a methodological study to determine the extent to which our observational instrument was capable of distinguishing the unique characteristics of different preschool programs. Two model preschool programs with important differences in educational philosophy were compared. We studied three classrooms following the Montessori philosophy and three following the child-centered model. Some of the analyses now completed show differences between these programs in predicted directions. For example, children in the child-centered schools engaged in significantly greater fantasy play and show more self-expressive behavior; while the children in the Montessori classrooms spend more time on activities involving quantitative skills, visual discrimination, and reading and writing. We expect that the additional analyses planned will give further validation of our Inventory of Children's Preschool Experience.

A paper describing the observational method and discussing some of the methodological issues involved in the use of the instrument and in reduction of data from observational techniques was presented by Dr. Robert Klein on a symposium at the annual meetings of the American Psychological Association in Washington, D. C.

Professional activities of the staff have included membership on committees of professional societies, on the editorial boards of journals and as consultants to government and non-governmental organizations.

Frank Pedersen is serving on a committee of the Society for Research in Child Development which is attempting to formulate a code of ethical principles to guide research with young children and their parents.

Robert Klein is a member of an Interagency Task Force which is monitoring contracts evaluating H.E.W. programs for the handicapped. Frank Pedersen and Robert Klein have provided consultation to the Bureau of Standards regarding the development of methods to measure forces which children two to six years of age exert on play materials. This is in connection with the Bureau of Standards work in setting industry standards for toy safety.

Alice Abramson is serving on the Professional Advisory Committee of the Easter Seal Treatment Center.

Leon Yarrow continues to serve as a consultant to the Research Division of the Office of Child Development. He is also consulting with the Advisory Committee on Child Development of the National Research Council which is reviewing knowledge in critical areas of child health and development as a background for policy and program recommendations. He is continuing to serve on the Advisory Committee for Documentaries on Kibbutz Education of the Institute for Child Mental Health.

Dr. Yarrow is currently President of the Society for Research in Child Development and a member of the Long-range Planning Committee of the Society. He is also a member of the Executive Committee of the Society for the Study of Developmental Intervention. He continues to serve on the editorial boards of the Merrill-Palmer Quarterly Journal of Human Development, and as editorial consultant to the Monographs of the Society for Research in Child Development and the Public Health Reports.

Dr. Stuart Copans, Dr. David Schachter and Dr. Bradley Soule III became members of the staff in July 1971 as Research Associates. Dr. Colleen Rand and Ms. Penny Fisk joined the staff as guest workers in the past year. Dr. Walter Brown and Dr. Gordon Keating completed their assignments as Research Associates in June 1971. Dr. Joan Bissell, Staff Fellow, resigned. Ms. Florine Carpenter entered on duty as secretary to replace Ms. Joanne Mechler.

Papers Presented at Professional Meetings:

Stuart Copans. "Human Prenatal Effects: A Suggested Methodology for Future Study," Southeastern Conference on Research in Child Development, April 20-22, 1972, Williamsburg, Virginia.

Robert Klein. "An Approach to the Measurement of Preschool Environments," Annual Meeting of American Psychological Association, September 1971, Washington, D. C.

Frank Pedersen, Leon Yarrow and Judith Rubenstein. "Tactile-Kinesthetic Stimulation in Infancy," Southeastern Conference on Research in Child Development, April 20-22, 1972, Williamsburg, Virginia.

Leon Yarrow. "Enrichment and Deprivation: Towards a Conceptual and Empirical Differentiation of the Early Environment," Symposium on Enrichment and Deprivation at meeting of International Society for the Study of Behavioral Development, July 8, 1971, Nijmegen, The Netherlands.

Leon Yarrow. "Some Issues in Studying Mother-Infant Interactions," Seminar on the Effect of Home Environment and Early Social Development on Cognitive Growth and the Transition to Formal Education sponsored by the Social Science Research Council, at the Center for Advanced Studies in the Behavioral Sciences, December 6, 1971, Stanford, California.

Publications:

Brown, W. A. Seven women: A prospective study of postpartum psychiatric disorders. Psychiatry, In press.

Burnes, K. Clinical assumptions about WISC subtest score and test behavior relationships. Journal of Consulting and Clinical Psychology, 1971, 36, 2.

Burnes, K., Brown, W. A., & Keating, G. W. Dimensions of control: Correlations between MMPI and I-E scores. Journal of Consulting and Clinical Psychology, 1971, 36, 301.

Yarrow, L. J., & Pedersen, F. A. Attachment: Its origins and course. In W. W. Hartup (Ed.), The Young Child. Washington, D. C.: National Association for the Education of Young Children, 1972, Pp. 54-66.

Yarrow, L. J., Rubenstein, J. L., Pedersen, F. A., & Jankowski, J. J. Dimensions of early stimulation and their differential effects on infant development. Merrill-Palmer Quarterly, 1972, 18, In press.

Yarrow, L. J., Goodwin, M.A., Manheimer, H., & Milowe, I. D. Infancy experiences and cognitive and personality development at ten years. In L. J. Stone, H. T. Smith & L. B. Murphy (Eds.), The Competent Infant, Basic Books, In press.

Yarrow, L. J., & Goodwin, M. S. The immediate impact of separation: Reactions of infants to a change in mother-figures. In L. J. Stone, H. T. Smith, & L. B. Murphy (Eds.), The Competent Infant, Basic Books, In press.

Serial No. HD-SB4(c)
1. Social and Behavioral Sciences Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Effects of Infant Temperamental Characteristics on Mother-Infant Adaptation

Previous Serial Number:

Principal Investigator: Leon J. Yarrow, Ph. D.

Other Investigators: Stuart Copans, M.D., William Davidson, M.D., Robert Klein, Ph. D., Frank Pedersen, Ph. D., Bradley Soule, M.D., Kay Standley, Ph. D., Colleen Rand, Ph. D., Alice Abramson, M.A., Richard Cain, M.Ed., Myrna Fivel, M.A.

Cooperating Units: Pediatrics and Obstetrical Services of National Naval Medical Center

Man Years:

Total:	5.75
Professional:	5.50
Other:	0.25

Project Description:

Objectives: To study the effects of infant temperamental characteristics on patterns of maternal care and attitudes towards the infant. Within this overall objective there are a number of specific methodological and substantive questions:

1. Is there a basic consistency between infant characteristics assessed in the neonatal period and infant characteristics at four weeks.
2. What is the relationship between characteristics of infants, e.g., cuddliness, consolability, responsiveness to animate and inanimate stimuli assessed in the neonatal and four week period and the mother's perceptions of her infant.
3. What are the relationships between mother-infant adaptation at four weeks and certain prenatal variables: her prenatal attitudes and expectations regarding the infant; the husband-wife relationship during pregnancy; the woman's physical and psychological adaptation to the pregnancy experience.

4. To what extent is there an interaction between maternal personality characteristics and infant temperamental characteristics in influencing maternal caretaking patterns and mother-infant adaptation. For example, does a mismatch in activity level between mother and infant lead to disturbed adaptation; is there a relationship between the infant's visual alertness and amount of visual stimulation provided by the mother.

Methods: The sample will consist of 40 mothers and their first-born infants, 20 boys and 20 girls. Only normal infants, free from complications during pregnancy and delivery will be included in the study.

The core design involves assessing infant characteristics during the neonatal period using the Brazelton Neonatal Assessment Scale and again at four weeks of age, and studying mother-infant interaction in detail at four weeks. In addition a recruitment interview will be conducted prenatally with the mother and father in which basic demographic data will be obtained, information about their adaptation to the pregnancy experience, the degree of support given the wife by the husband, and both parents' expectations and feelings toward the unborn child. The mother will be interviewed again when the infant is four weeks old.

Major Findings: Data collection is now in progress. An observation scale was designed to assess mother-infant interaction at four weeks. It includes categories of stimulation in the auditory, visual, tactile and kinesthetic modalities, measures of contingent responsiveness to the infant in each of these modalities and measures of affective contact. Good reliability has been obtained among three observers on the major categories of mother-infant observation. Excellent interobserver reliability has also been established among three investigators on the Brazelton Neonatal Assessment Scale. An interview has been developed to assess selected maternal personality characteristics, her feelings about the maternal role and her perceptions of the infant's characteristics.

Significance to Biomedical Research and the Program of the Institute: Most research on early development has concentrated on environmental influences on the young infant conceptualized in essentially unidirectional terms, i.e., the effects of environmental events on infant development. This model is being replaced by an interactional one in which the child is seen as an active agent who influences the behavior of other people towards him.

This investigation should begin to give some understanding of reciprocal interactions between infant and mother.

Proposed Course of Project: Collection of data will be continued during the next fiscal year.

Honors and Awards: None

Serial No. HD-SB2(c)

1. Social and Behavioral Sciences Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: An Observational Study of Father-Infant Interaction

Previous Serial Number: HD-SB2(c)

Principal Investigators: Frank A. Pedersen, Ph. D., David Schachter, M.D.

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	1.5
Other:	

Project Description:

Objectives: The objectives of this study have been modified since the previous project report. Its focus is now on the very early period of the developing father-child relationship.

The major objective is to obtain descriptive data on fathers' interactions with first-born infants. Among the questions in which we are interested are: the ways in which fathers differ in their interactions with male and female infants; how fathers differ from mothers in the quality of stimulation and affectionate contact they provide boy and girl infants; and whether the father's relationship with the baby affects the mother's behavior towards the infant. In addition we will obtain some data on an important methodological problem--the comparison of self-report and observational data.

Methods Employed: The sample will consist of approximately 40 middle-class fathers and their first-born infant (the same sample used in Project HD-SB4(c). At age four weeks the father is interviewed regarding the early postnatal period, his current relationship with the infant, and aspects of the husband-wife relationship which may bear on how each parent relates to the baby. At age five weeks an observation is conducted in the home, usually in the early evening, focusing on the father's interaction with the baby. The observational system is an adaptation of the method described in Project HD-SB4(c). Data from that project on the infant and the mother-infant relationship will be utilized in certain analyses.

Major Findings: The change in focus of this investigation is the result of a number of considerations. The earlier study, involving videotape observations of mother-father and 2½ year child interaction and narrative records of playroom behavior, required very difficult, time-consuming coding methods. After completing a small number of pilot subjects, we had an uneasy feeling that there were many important aspects of the way the father-child relationship develops that could not be understood with a simple cross-sectional approach in the preschool years. Practical considerations, including personnel shortages also entered into the decision to reshape the study.

In focusing on the earliest periods of the father's contact with the baby, we have attempted to conceptualize the role of the father in terms of three areas: (1) his behavior in direct interaction with the baby; (2) his behavior which is instrumental to the mother, such as assisting in various household activities; and (3) his emotional support given to the mother during her early adaptation to the baby. We have developed an interview that covers these areas. Reliability of ratings made from tape recordings appear very good. The adaptation of a mother-infant observational system to focus on the father's behavior with the baby has been completed. Observer agreement on behavioral categories was originally established with mothers and is now being extended to fathers as well. We are impressed with the wide range of differences in fathers in all areas, but it is too early in the data collection process to give more than impressionistic results.

Significance to Biomedical Research and the Program of the Institute: Most of our knowledge of early parental behavior is limited to the mother-infant unit. The apparent assumption that this is a closed system, relatively unaffected by the father, and that early paternal behavior is of no consequence is at least open to question. The observational data of father-infant interaction will be unique in their own right, and it is hoped that this study will contribute to our understanding of the infant's early environment.

Proposed Course of Project: It is expected that data collection will continue through the summer months. Initial coding and tabulations should be completed in the coming year.

Honors and Awards: None

Serial No. HD-GD4 (c)

1. Social and Behavioral Sciences Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Environmental Influences on Cognitive and Personality Development in Infancy

Previous Serial Number: Same

Principal Investigators: Leon J. Yarrow, Ph. D., Judith L. Rubeinstein, Ph. D., Frank A. Pedersen, Ph. D.

Other Investigators: Myrna Fivel, M.A.

Cooperating Units: D. C. Department of Public Health, Children's Hospital of the District of Columbia, Group Health Association of Washington, D. C.

Man Years:

Total:	1.75
Professional:	1.25
Other:	.50

Project Description:

Objectives: In this investigation of early environmental influences on cognitive and personality development during the first year of life, one major goal has been to develop an observational instrument to measure differentiated aspects of the early environment. A second objective was to study relationships between specific parameters of the environment and specific aspects of development during the first six months.

Methods Employed: The main sample, composed of 55 infants and their caretakers from low socio-economic backgrounds, were obtained through two well-baby clinics in the District of Columbia. To extend the range of environmental variables and to permit some exploration of the effects of social class status on early maternal care, this group was augmented by a sample of 15 infants from middle-class backgrounds obtained through a private medical care program. Pediatric and neurological examinations were conducted to exclude infants with abnormalities that might be associated with developmental retardation.

Data on environmental variables were obtained through time-sampling observations in the home when the infants were five months of age. Measures of infant characteristics were obtained by means of the Bayley Scales of Infant Development and a situational measure of exploratory behavior and preference for novel stimuli. In addition, clusters were developed from the items on the Bayley Scales as measures of selected social, cognitive and motivational functions: social responsiveness, goal orientation, object permanence, secondary circular reactions, gross motor functions, fine motor functions, visual discrimination, reaching and grasping, and language.

Major Findings: In previous analyses we found relations between dimensions of the social and inanimate environment and some specific developmental functions, e.g., early goal-directed behavior, the beginnings of object permanence and secondary circular reactions. Further analyses of social stimulation have been carried out from a different perspective. We have analyzed social stimulation in terms of specific sensory modalities--visual, auditory, tactile, and kinesthetic--and looked at their relationship to infant development. These analyses indicate that kinesthetic stimulation, i.e., rocking, jiggling the baby's body in play, transporting him about the house, is an especially important modality of stimulation. Amount of kinesthetic stimulation is significantly related to the infant's mental and motor developmental status, the degree of social responsiveness the infant shows and such cognitive-motivational functions as goal orientation, secondary circular reactions and object permanence. Other modalities of stimulation showed more circumscribed relationships with infant development. For example, distance receptor stimulation was found to be related to social responsiveness alone. Although tactile and kinesthetic stimulation are sometimes equated as part of the infant's "contact needs," our results emphasize the value of distinguishing these modalities. Experimental studies with primates and human neonates have pointed to a special significance of kinesthetic stimulation. Our data extend these findings to the natural environment.

More detailed analyses of the data show that the significance of different modalities of stimulation is dependent on the sex of the infant. On the whole, we find many more correlations which are significant or of a higher magnitude for female infants. The few significant relationships between environmental variables and the development of male infants are consistent with findings from other studies. For example, visual stimulation (indexed by the amount of mutual visual regard between mother and infant) relates significantly to social responsiveness in males while auditory stimulation (talking directed to the baby) relates to social responsiveness in girls. We also find that the stimulation provided by the mother seems to be affected by the sex of the infant; male infants tend to receive more social stimulation through all modalities than female infants. In order to understand the differential relationships with environmental variables, it seems necessary to postulate complex interactions between biologically determined sensitivities and patterns of maternal behavior.

We have also performed multivariate analyses in an attempt to deal with more complex questions such as whether certain environmental variables are

interchangeable, whether certain combinations of variables are additive in their effects, and to what extent certain variables interact to strengthen or diminish each other's impact. In general, we find a severe grossly depressing effect of environments which are low on both social and inanimate stimulation. However, we find no evidence of statistical interactions that indicate an enhancing effect of high social and high inanimate stimulation; that is, we have not found particular combinations of variables associated with vastly accelerated development.

A paper reporting the results of the different modalities of stimulation and the sex differences was recently presented at a professional meeting. A condensation of four papers presenting previous statistical results is in press and will also be incorporated into a book of readings on infant development.

Significance to Biomedical Research and the Program of the Institute: In addition to the potential significance of the findings of this research for theories of early development, we expect that the techniques developed for home observations will be useful contributions to methodology.

Proposed Course of Project: It is planned to report the major findings in monograph or book form and terminate this study in the next fiscal year.

Honors and Awards: None

Related Publications: Yarrow, L. J., Rubenstein, J., Pedersen, F. A., and Jankowski, J. J.: Dimensions of Early Stimulation and Their Differential Effects on Infant Development. Merrill-Palmer Quarterly, 1972, 18, 34-41.

Serial No. HD-SB3(c)
1. Social and Behavioral Sciences Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Assessment of Infant Temperament

Previous Serial Number: Same

Principal Investigator: Leon J. Yarrow, Ph. D.

Other Investigators: William Davidson, M.D., Kay Standley, Ph. D., Robert P. Klein, Ph. D., Kay Jennings, M.A., Alice Abramson, M.A.

Cooperating Units: Pediatrics and Obstetrical Services of National Naval Medical Center

Man Years:

Total:	1.00
Professional:	0.75
Other:	0.25

Project Description:

Objectives: This was essentially a methodological study. Its primary purpose has been to develop measures of basic temperamental characteristics in young infants which could be used in a subsequent investigation of infant effects on maternal behavior. Because of their salience for maternal behavior, attention was focused on the infant's social responsiveness and ease of being soothed. The stability of these temperamental characteristics over the first five months of life was assessed.

Methods Employed: The sample consisted of 38 first-born, healthy infants from mothers with normal pregnancies and deliveries. Subjects were obtained through the Obstetrics Department at the National Naval Medical Center.

Each infant was studied at one, three, and five months of age, with two sessions at each age level in order to control day-to-day fluctuations in behavior. The infant's reactions to a standardized series of social and inanimate stimuli, presented while in a content state and while in an irritable state, were recorded. In addition to these structured observations, the mother's perceptions of her infant's characteristics were obtained by interview.

Major Findings: Data collection and a major share of data analysis have been completed.

The initial concern was the degree of observer reliability for measures, particularly for ease of soothing, which seemed most vulnerable to subjectivity. Observer reliability was judged satisfactory (78%).

The next step was the determination of inter-session reliability (i.e., consistency) of the measures at each age level. The order of stimulus presentation was reversed during the second session. Generally, the degree of inter-session reliability was fairly low, but beyond chance expectations. Social responsiveness showed somewhat higher inter-session consistency than ease of soothing, which had more marked order effects.

The major part of the analysis focused upon the degree of stability across the three age periods. For this purpose, the scores obtained from the two sessions at each level were averaged for individual subjects. The number of significant correlations between different age levels was only slightly greater than that expected by chance. Therefore, it was concluded that ease of soothing and social responsiveness (as measured) were not stable infant temperamental characteristics during the first five months of life.

The final part of the analysis, now in progress, is focused upon changes in infant reactions to the specific stimuli.

Information gained from the infant observations and from the maternal interviews has provided ideas for important variables affecting mother-infant interactions and for the development of new measures of these variables.

Significance to Bio-medical Research and the Program of the Institute: In studies of mother-infant relationships, the impact of the mother upon her infant has generally been emphasized. Recently, there has been increased concern with the potential effect of the infant's temperamental characteristics upon maternal behavior. An important first step towards understanding the infant's role is the delineation of basic temperamental characteristics. Finding stable characteristics and/or defining changes in a period of rapid development is a formidable task, but success in this endeavor should lead to a better understanding of the factors shaping the mother-infant relationship. The information obtained from the infant observations and the maternal interviews has been useful in the development of measures for a study now underway of infant effects on maternal behavior.

Proposed Course of Project: Analyses will be completed and study terminated during this fiscal year.

Honors and Awards: None

Serial No. HD-GD3 (c)

1. Social and Behavioral Sciences Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Effects of Preschool Experiences on the Intellectual and Personality Development of Children from Culturally Deprived Backgrounds

Previous Serial Number: Same

Principal Investigator: Leon J. Yarrow, Ph. D.

Other Investigators: Robert P. Klein, Ph. D., Frank A. Pedersen, Ph. D., Kay Standley, Ph. D., Sandra Sklar, M.A., Bette Marcus, M.A.

Cooperating Units: Office of Economic Opportunity, Project Headstart, National Capital Day Care Association

Man Years:

Total:	1.25
Professional:	1.00
Other:	.25

Project Description:

Objectives: This study has been concerned with the impact of early preschool experiences on the development of cognitive skills, behavioral controls and self-concept in four-to-five-year-old children from culturally disadvantaged backgrounds. A major goal has been to develop methods for differentiated analyses of preschool learning environments, utilizing concepts from social learning theory, cognitive developmental theories and operant learning theory.

Methods Employed: The sample consisted of 58 children in 12 preschool classrooms in Headstart and day care programs. Time-sampling observational techniques were used to analyze the preschool environment. To provide a sample of different classroom activities and minimize effects of day-to-day changes, each child was observed for 20 observational units on each of three days in a balanced order. These observations were made on each child at three different points in the school year--in the fall, shortly after the beginning of school; in the middle of the term; and in the early summer,

toward the end of the term. A comprehensive observational system was developed. This system is organized in terms of the following major categories: teacher behavior, which include dimensions of teacher control, cognitive input dimensions, the affective qualities of the interaction, and evaluative feedback; child-behavior variables, focusing on self-initiated or self-directed activities; and background and setting variables.

Measures of the dependent variables consisted of several standard tests and others developed especially for this investigation. For studying cognitive functions, the following techniques were used: the Wechsler Preschool Scale of Intelligence, several measures of language functions, the Johns Hopkins Perceptual Test, selected items from the Frostig Developmental Test of Visual Perception, and a specially designed object categorization test for assessing concept development. As measures of "behavioral controls," we have adapted the Maccoby motor inhibition measures, and have developed techniques to assess capacity to delay gratification, a measure of focused attention, and a situational test of response to a difficult task. In addition, we have developed a self-concept test designed to tap various aspects of the child's feelings about himself, his competencies and deficiencies, and his perception of how he is evaluated by other people.

Major Findings: Analyses of the data have been completed. To assess the relationships between the child's experiences in the classroom and changes in general intellectual level, in specific cognitive functions, and on his self-concept simple correlational analyses and multiple regression analyses were carried out.

In general, the amount of cognitive stimulation in these preschool settings as measured by time spent on auditory discrimination tasks, activities involving categorization, quantitative skills, etc. was very small. Somewhat more time was spent on visual discrimination and labeling activities.

We analyzed many relationships between specific cognitive inputs and changes in the children's performance on specific tests, e.g., the relation between the amount of time spent on labeling and changes in scores on vocabulary tests, the amount of visual discrimination training and changes in scores on tests of perceptual discrimination. On the whole, we found few significant relationships, no more than can be expected by chance. These findings of few measurable effects tend to be consistent with several other studies of the impact of compensatory preschool programs.

During this year we have revised the observation manual, clarifying the definitions of the categories in order to increase its usefulness to other investigators.

A paper describing the observation method was presented at the 1971 meeting of the American Psychological Association by Dr. Robert Klein as part of a symposium, Observational Methods for Studying Preschool Environments, organized by Dr. Leon Yarrow.

Significance to Biomedical Research and the Program of the Institute: Current controversy about the relative merits of various approaches to preschool programs emphasizes the need for greater conceptual clarity about the preschool environment. The concepts and techniques developed in this research should contribute to more adequate methodologies for studying complex environmental inputs in relation to the cognitive and personal-social development of preschool children.

Proposed Course of Project: This investigation will be completed during the next fiscal year. A publication is planned which will include a discussion of the conceptual bases for the choice of observation categories, a discussion of some methodological issues in the use of observation techniques and issues regarding reduction of variables for statistical analyses. The detailed procedures for use of the observation manual will be included in this report.

Honors and Awards: None

Serial No. HD-SB1(c)

1. Social and Behavioral Sciences Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: An Observational Approach to the Measurement of Preschool Environments

Previous Serial Number: Same

Principal Investigator: Leon J. Yarrow, Ph. D.

Other Investigators: Robert P. Klein, Ph. D., Sandra Sklar, M.A., Alice Abramson, M.A.

Cooperating Units: Montessori Society of Chevy Chase, Bank Street College of Education

Man Years:

Total:	0.75
Professional:	0.75
Other:	

Project Description:

Objectives: This research is concerned with refinement and further development of a time-sampling observational technique for describing children's experiences in preschool settings. The observational technique was developed in an earlier study to assess the impact of experiences in Headstart programs. In this study two model preschool programs with differences in educational philosophies were compared. We were interested in determining the extent to which the observational instrument was able to distinguish unique characteristics of different kinds of preschool programs. A second and related purpose of the study is to describe differences in children's experiences in programs based on different philosophies. Although there has been a proliferation of models for preschool intervention during the past several years, there is as yet no observational research describing the ways in which these programs vary in terms of children's experiences.

Methods Employed: The sample consisted of 48 children in six preschool classrooms, three based on the Montessori 'prepared environment' model and three on the Bank Street College 'child-centered' model. To provide a representative sample of the different classroom experiences of children in the two models, each child was observed for ten observational units of two

minutes duration on each of six days. Our previously developed observational technique was refined for use in this study. The observational scheme describes the following dimensions: child-behavior variables, focusing on the nature of a child's cognitive, perceptual-motor, language, social and affective experiences; teacher-behavior variables, including components of teacher control, cognitive input, affective interaction with children, and evaluative feedback; and structural components of the environment, including the degree of structure of classroom materials and the extent of freedom given the child in choice of activities.

Major Findings: Basic counts of the observation categories have been obtained as well as counts of more complex variables which are combinations of the observation categories. A major focus of our efforts has been to derive predictions from the literature describing the philosophy of the Bank Street and Montessori programs. Since the articles discuss the programs in terms of abstract goals rather than in terms of specific teacher and child behaviors, we have consulted with representatives of the programs to check our interpretations of the literature. We then translated program philosophy into predictions in terms of category definitions. Several statistical comparisons of the programs have been completed using analysis of variance. The comparisons show that the scale is differentiating between the programs on predicted dimensions. For example, there is a significantly greater amount of fantasy play and self-expressive behavior of the children in the Bank Street Schools. In the Montessori classrooms there is significantly more time spent on pre-academic activities, such as, reading, writing, quantitative skills and in visual discrimination.

Significance to Biomedical Research and the Program of the Institute: Controversies about the relative merits of various approaches to preschool education and intervention have existed for several years. A major factor preventing resolution of these controversies is the lack of descriptive data based on direct observation of these different programs. The instrument refined in this project should provide a tool for further observational research. The substantive findings from the project should provide important data about the differing experiences of children in various programs.

Proposed Course of Project: The counts and tests of statistical significance should be completed early in the next fiscal year. Following these univariate analyses, we plan several multivariate analyses to determine which dimensions are most important in distinguishing the programs.

Honors and Awards: None

Annual Report
July 1, 1971 through June 30, 1972
Reproduction Research Branch
National Institute of Child Health and Human Development

SUMMARY

The mission of the Reproduction Research Branch is the development of scientific data and theory basic to reproductive biology. Research efforts therefore extend from the chemistry of the protein and steroid hormones to physiologic and clinical studies in man. To prosecute these investigations successfully, a variety of tools and skills are necessary: physical chemistry of macromolecules, protein isolation and analysis, tissue and organ culture, ultrastructure, enzymology, radioligand assays of protein and steroid hormones, mathematical analyses of immunoassays and the kinetics of protein hormone binding, characterization of *in vitro* steroid biosynthetic pathways, dynamic analysis of steroid hormone metabolism in man. A continuing interest and involvement in clinical problems are not only desirable but necessary since the results of study in man are the final arbiter of the relevance of results in the laboratory.

The group in protein chemistry has continued to develop and improve protein separation techniques. This has included design, fabrication, and testing of new instruments. Mathematical analysis of new systems has provided insights into molecular sieving techniques and has enabled investigators to predict conditions necessary for optimal resolution of pairs of proteins. This group collaborates extensively with scientists in Bethesda and around the world. Their expertise has placed them in an advisory capacity to many who need to use techniques developed in this laboratory. The section will be strengthened by the addition of Dr. Harvey Pollard, who has spent this year at Cambridge learning those techniques that have been developed for reconstructing lipoprotein membrane structure from electron photomicrographs.

There have been two major accomplishments in the tissue culture laboratory. Hybrid comatic cell lines were created by fusing choriocarcinoma cells with human and mouse fibroblasts. Several of the hybrid lines synthesized chorionic gonadotropin, showing for the first time that specialized functions can persist in hybrid somatic cells. The porcine granulosa cell maintained in tissue culture has proved a valuable tool. It was found that estrogens are potent stimulators of progesterone synthesis and that protein synthesis is necessary for this effect.

The Branch has continued its major contributions to research on gonadotropins. Antisera specific to subunits of human gonadotropins and to subunits of gonadotropins of other species have been prepared. It has thus been possible for the first time to measure small amounts of human chorionic gonadotropin in the presence of luteinizing hormone. As a result of this, pregnancy can be detected as early as the 5th post-ovulatory day, and ectopic production of chorionic gonadotropin by a series of human neoplasms has been

uncovered. The test for chorionic gonadotropin is also useful for monitoring the response to therapy of women with trophoblastic neoplasms. A patent for this test has been applied for. The use of the specific antisera has shown that within species immunologic cross-reactivity resides in the α-subunit, whereas between species cross-reactivity is due to the β-subunit.

An extensive mathematical treatment of the theory of radioimmunoassays and immunoradiometric assays (labeled antibody assays) was developed and validated experimentally. These studies and the application of the theory to obtain improved methods for data processing of radioimmunoassays have found use in many laboratories around the world.

In a series of other studies bearing on the gonadotropins, a radioligand receptor system for LH and HCG has been developed using the rat testis. The specific binding sites are associated with membranes and have high affinities. Functional coupling of the binding sites to the adenyl cyclase system was demonstrated. Using modified chorionic gonadotropin it was possible to dissociate binding from cAMP and testosterone production. The sensitivity of this system to gonadotropins means that it will be an important tool both for studying the actions of gonadotropins and attempting to inhibit their effects.

New radioimmunoassays were developed for several steroids-cortisol, dehydroepiandrosterone, androstenediol, 17-hydroxypregnenolone, and estriol. The resultant ease and sensitivity of analysis facilitated studies of adrenal and gonadal function. Dehydroepiandrosterone was shown to be a more important adrenal secretory product than its sulfate. Dehydroepiandrosterone was found to be secreted by ovary and testis, and became a major secretory product during stimulation. As part of these studies it was proved for the first time that glucocorticoids can affect ovarian steroid secretion, a finding that will necessitate reappraisal of several functional tests of ovarian activity.

Clinical investigation has continued as a prominent part of the research. An active male fertility clinic has been in operation for a year. Data obtained from these patients have confirmed the hypothesis proposed last year that seminiferous tubule factors are important in the regulation of pituitary secretion of follicle-stimulating hormone. In women, the diagnostic studies of patients with primary and secondary amenorrhea have given information about syndromes of hypogonadotropic hypogonadism and of several types of secondary amenorrhea. As part of related endocrinologic interests, the endocrine profile of patients with Hand-Schuller-Christian disease has been delineated and a subtle type of hyperadrenocorticism (micronodular hyperplasia) causing severe osteoporosis has been described.

The Section of Reproductive Anthropometry will be disbanded this year due to the retirement of the section head. The unique data obtained over 35 years and available in the tape at the University of Minnesota will be used through a contract. Dr. Alan Treloar will continue to direct the contract work on a part-time basis.

Our labors this year have again been aided greatly by visiting scientists, fellows, and guest workers. These scientists and our Clinical Associates have been a continued source of stimulation. The collaborative efforts within the Branch and with other scientists in Bethesda and elsewhere have strengthened the research and multiplied our effectiveness. Finally, the support of the Institute for the use of service contracts has enabled us to shift some of the burden of routine work and has thereby freed our staff for more creative research.

NICHD Annual Report
July 1, 1971 through June 30, 1972
Reproduction Research Branch
Section on Endocrinology

A Summary Statement

Effects of Human Gonadotropins on Non-human Primate Testis Function and Morphology:

The action of human menopausal gonadotropin (luteinizing hormone and follicle-stimulating hormone) on testis hormone secretion, spermatogenesis and Leydig cell morphology was studied in immature rhesus monkeys. The hormone was administered in increasing doses. The low testosterone levels increased to adult levels during HMG treatment. Testis biopsies before and during treatment showed hypertrophy and increased vacuolization of Leydig cells. Initiation of spermatogenesis and maturation of spermatozoa were also observed. After six weeks treatment, plasma testosterone levels declined and antibodies to human luteinizing hormone (hLH) were demonstrable in some animals. These are the first quantitative data of the response of the immature primate testis.

In acute studies on the effects of human gonadotropins on the regulation of testosterone (T) secretion by rhesus monkey testis, it was found that testosterone levels rose about two-fold after 30 minutes infusion with human chorionic gonadotropin (1000 IU/hr) both in immature and mature animals. Elevated levels were maintained until experiments were terminated at two hours after completion of a two-hour infusion. Results from one prepubertal animal indicate a similar rapid increase of T secretion following infusion of hLH (1000 IU/hr).

Pregnancy in the Rhesus Monkey:

Hormonal changes during pregnancy were studied in ten rhesus monkeys. Plasma estradiol-17β levels were elevated just before ovulation, at 20 days of gestation and after reaching a plateau, from about 100 days until term. Plasma estradiol levels during pregnancy have not been described previously. Plasma progesterone levels also increased at mid-gestation with a slight decrease during the week preceding parturition. The decline in plasma progesterone levels is in agreement with a recent investigation of a series of human pregnancies.

Urinary estrone levels increased about three-fold during the last two weeks of gestation and declined sharply during the 3 days preceding delivery. The sharp pre-term decline in urinary estrone may provide a useful indicator of impending delivery.

Chimpanzee Chorionic Gonadotropin:

Urinary chorionic gonadotropin from two pregnant chimpanzees was quantified during middle and late gestation. Both biological and radioimmunological assays were employed, as well as two methods of precipitation of gonadotropic hormones. The patterns of excretion, and the immunological and biological properties of chorionic gonadotropin in the chimpanzee and man are remarkably similar. The development of methods for detection of chorionic gonadotropin in this non-human primate provides a useful parameter for experimental investigation of pregnancy in an animal closely related to man.

Primate Gonadotropins:

Neutralization of the biological and immunological activities of primate chorionic gonadotropins by antisera to the α and β subunits of hCG was compared. The relative antigenic similarity of sub-human primate chorionic gonadotropins to hCG was consistent in both bioassay and radioimmunoassay. Gorilla chorionic gonadotropin activity was more effectively neutralized by antisera to both the α and β subunits of hCG than was chimpanzee chorionic gonadotropin. These same antisera had no demonstrable neutralizing effect on rhesus monkey chorionic gonadotropin in either bioassay or radioimmunoassay.

Male Excurrent Ducts:

The total glycerol concentrations in the excurrent duct tissues of the adult male rat were found to have a characteristic pattern similar to the concentrations of glycerylphosphorylcholine in fluids collected from the testis, epididymis and vas deferens. Rats given sufficient doses of 3-chloro-1,2-propanediol (chlorhydrin) to cause infertility had normal total glycerol concentrations in testis, epididymis and vas deferens tissues suggesting that the chlorhydrin does not act as a metabolic analogue of glycerol in excurrent duct tissues of the rat.

The rates of incorporation of labeled serotonin and its precursor 5-hydroxytryptophan into excurrent duct tissues of the adult male rat were compared *in vitro*. Testis parenchyma preferentially accumulated 5-hydroxytryptophan. On the other hand, the vasculature supplying the testis selectively accumulated serotonin. These data suggest that preferential uptake of either indoleamine by male reproductive tract tissue may reside with its inherent density of sympathetic neurons.

Maturation of Testicular Enzyme Systems:

The specific activities of three enzyme systems in rat testis have been studied during the maturation process up to 90 days of age. A two-fold rise in gamma glutamyl transpeptidase (γ-GT) occurred between 15 and 20 days of age; after which the enzyme's concentration was constant through 90 days of age. Histochemical localization of gamma glutamyl transpeptidase and its increased concentration in atrophied testes of hypophysectomized rats suggest that this enzyme may be localized in non-germinal cells of the seminiferous tubule.

The specific activity of sorbitol dehydrogenase (SDH) increased in parallel with the increased growth rate of the testis. Both these enzymes increased markedly at 20 to 25 days of age and may be indicative of the initial, sustained endogenous testosterone production, just preceding the initial meiotic division.

Total lactic dehydrogenase (LDH) activity in rat testis declined steadily from 5 to 20 days of age before increasing at 27 days of age. This increase occurred simultaneously with the initial detection of LDH-x and was temporally associated with the histological appearance of secondary spermatocytes.

Treatment of rats hypophysectomized at 18 days of age with 2 IU of Pergonal (FSH and LH) daily for 7 or 14 days stimulated testis growth at nearly the same rate as intact, control rats. However, the metabolism of these testes was apparently abnormal, since the specific activities of LDH, SDH and γ-GT were not within normal limits when Pergonal (1 or 2 IU daily) and/or hCG (5 IU daily) was administered for 7 or 14 days.

Rat semen, collected from normal adult rats by electroejaculation, con tained LDH-x exclusively and was absent from ejaculates of vas-ligated rats, thereby demonstrating its localization in mature sperm cells.

Ultrastructure:

Changes ir annulate lamellae were correlated with other cytoplasmic and nuclear events related to cell division. In prophase, the condensing chromosomes become attached to newly-forming intranuclear annulate lamellae. During late prophase and in early metaphase both, the pronuclear envelopes and the annulate lamellae undergo similar alterations. Pores disappear from both of them and the remaining cisterne coalesce. No annulate lamellae were observed after complete breakdown of the pronuclear envelopes. During late anaphase, small vesicles of the smooth endoplasmic reticulum abut to individual chromosomes, then the chromosomes extend pseudopod-like structures, karyopods. These serve as bridges during the union of the individual membrane-bounded chromosomes into a condensed nucleus. Annulate lamellae are reformed during late telophase and early interphase from paired and multilamellar cisternae. Thus, it was established that in the rabbit zygote annulate lamellae disappear during cleavage and they are reformed from stored components after cleavage. Also, new annulate lamellae are formed after cleavage by fusion of vesicles which originate from the nuclear envelope.

Microfilaments were noted directly under the cell membrane at the leading portions of cleavage furrow in the rabbit zygotes. These filaments are parallel with the cell membrane at either side of the furrow and are perpendicular to the membrane within the leading portion of the furrow. These filaments were identified as the morphological entity of the contractile ring in the dividing zygotes. Stem bodies are spindle microtubules which are embedded in an electron dense matrix. The stem body becomes associated with the microfilaments of the cleavage furrow and at completion of cleavage, it becomes the mid-body.

All animal cells are believed to possess centrioles which serve as focal points at the two opposite ends of the spindle and the spindle microtubules emerge upon them. It was discovered that the rabbit zygotes lack centrioles, and instead they have several fibrillar aggregates which serve as microtubular organizers. Thus, the centrioles of the fertilizing sperm do not become the centrioles of the zygote as it has been thought until now. Centrioles appear in the cells of the morulae. Lastly, the mammalian zygotes may represent a link between the cell division apparatus of plant cells, which lack centrioles, and animal cells.

Serial No. RPL-1
1. Reproduction Research Branch
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Biology of Reproduction: Clinical and Experimental

Principal Investigator: Dr. Mortimer B. Lipsett

Other Investigators: Dr. G. T. Ross, Dr. W. W. Tullner, Dr. P. O. Kohler, Dr. C. M. Cargille, Dr. W. Bridson, Dr. R. J. Sherins, Dr. A. Treloar, Dr. D. Rodbard, Dr. A. Chrambach, Dr. H. Pollard, Dr. K. Catt, Dr. M. D. Catt, Dr. H. Kulin, Dr. B. Gulyas, Dr. W. Nixon, Dr. G. Hodgen, Dr. E. Reiter, Dr. R. Goldenberg, Dr. J. Grodin, Dr. H. Ruder, Dr. D. L. Loriaux, Dr. G. Braunstein, Dr. T. Tsuruhara, Dr. G. Kapadia, Dr. R. Becker, Dr. P. Krueger, Dr. B. Nisula, Dr. R. Mecklenburg, Mr. P. E. Rayford, Mr. D. Ryan, Mr. G. Coffman, Mrs. M. Wyckoff, Mrs. I. Hellman, Mr. C. Turner, Mr. D. Barber, Mr. Rudolph Reid, Mr. J. Brice.

Cooperating Units: Veterans Administration Hospital, Palo Alto; Department of Applied Mathematics, Harvard Univ., Boston; Department of Anatomy, Harvard Univ., Boston; Univ. of Paris School of Medicine, Paris; Max Planck Inst., Gottingen; Columbia Univ. School of Medicine, New York; Cornell Univ. School of Medicine, New York; Georgetown Univ. School of Medicine, Washington, D.C.; Emory Univ. School of Medicine, Atlanta; Arctic Health Research Center, Alaska; M. D. Anderson Inst., Houston; Physical Sciences Laboratory, DCRT; Laboratory of Biochemistry and Metabolism, NIAMD; Laboratory of Mineral Metabolism, NIAMD; Laboratory of Developmental Immunology, NICHD; Laboratory of Experimental Pathology, NIAMD; Laboratory of Cell Biology, NCI; Laboratory of Biomedical Sciences, NICHD; Newark-Beth Israel Medical Center, Newark; Department of Obstetrics & Gynecology, National Naval Medical Center, Bethesda; Department of Obstetrics & Gynecology, George Washington Univ. Medical School, Washington, D. C.

Man Years:

Total: 55
Professional: 37
Other: 18

Project Description:

Objectives:

1. To understand the mechanisms of regulation of gonadal secretion;
2. To define the regulatory processes involved in maintenance of blood and tissue hormone levels;
3. To analyze the events of the normal and abnormal menstrual cycle;
4. To explore the control of spermatogenesis;
5. To study and attempt to modify the side-effects of estrogens.

Methods Employed:

A great variety of techniques are employed since the research encompasses such widely divergent areas as mathematical biology, protein hormone chemistry and physiology, tissue culture, steroid biochemistry, morphology, and clinical investigation. New methods that have been either developed or adapted during the year are:

Development of parameter-fitting programs for estimating affinity constants and describing radioimmunoassay systems in complex heterogeneous antigen-antibody systems.

Use of isoelectric focusing on polyacrylamide gel to give estimates of molecular size and identity. Development of high cross-linked gels and agarose-polyacrylamide gels for fractionation of high molecular weight species.

Production of specific antisera for protein chromosome subunits.

Development of a new enzymatic method for radioiodination of protein and peptide hormones.

Development of an agarose-concanavalin A column to separate glycoprotein hormones.

Major Findings:

I Protein Chemistry

Protein Hormones:

Human Prolactin: The physical characterization of serum hGH previously based on the Turkington assay was repeated, using analytical polyacrylamide gel electrophoresis (PAGE) and isoelectric focusing on polyacrylamide gel (IFPA) of serum hPR from a high titer serum in conjunction with the heterologous anti-porcine prolactin RIA. Serum hPR, as identified by this assay, is a molecule of slightly larger size than hGH-B to E and of less net charge than hGH-B. The apparent isoelectric point of hPR (defined by the RIA used) is 6.5. This accounts for the reported insolubility of pituitary hPR in water and will be of importance for the isolation of serum hPR.

Separation of the subunits of hCG: to obtain immunological specificity, it is necessary to separate the 3 antigens from each other quantitatively. Optimal resolution of hCG and of beta-subunit was obtained at pH 5.5. Here, each of the two molecules gives rise to at least 3 charge isomers. However, the least charged beta-subunit component and the most highly charged hCG component migrate in close proximity. Since the effective sizes of hCG and the beta-subunit are indistinguishable, IFPA is being used for this purpose.

The alpha-subunit behaves rather differently from the two other species. It has much lower anionic mobility, causing it to be retarded behind the stack in the buffer systems operative at pH 5.5 and 10.0; it is stacked, however, in a system operative at pH 11.0, together with the two other species. The second peculiarity of the alpha subunit is its strong adsorption to polyacrylamide. Adsorption in gels could be quantitatively reversed by polymerization of gels in the presence of 1 mg/ml bacitracin at pH 11. In lower pH systems, reversal is only partial.

Isolation of LHRH by PAGE: LHRH (Guillemin) after enzymatic iodination is heterogeneous in PAGE at pH 10; prior to iodination, only one component is detected after fixation and staining, but this may be due to our inability to date to fix some or all of the other components in any of a number of stain fixatives. A single component is biologically active and characterized by a molecular size at pH 10 compatible with that of a decapeptide. Two components of higher molecular weight appear to be aggregates of LHRH since they exhibit Ferguson plots that intersect with each other and with that of LHRH at the origin.

Isolation of bovine parathormone of increased specific activity: Increased specific activity of two bovine parathormone components has been obtained by IFPA. They are being fractionated further by PAGE and IFPA. Several components of each have been identified.

Methodologic Studies:

Polymerization efficiency: A method has been developed to measure the efficiency of conversion of acrylamide into polyacrylamide. This information is necessary to obtain an estimate of the reproducibility of polymerization, to optimize catalyst concentrations, and to permit valid interpretation of PAGE data on a quantitative basis.

Highly crosslinked gels: Polyacrylamide gels have been made, with up to 50% crosslinking (ratio of N,N'-Methylene-Bis Acrylamide to total monomer concentrations). These gels have unique properties, providing very large "pore" size, permitting application to multi-million molecular weight. These gels also make it possible to evaluate a number of predictions of theories regarding the pore structure and nature of polyacrylamide gels.

Isotachophoresis: i.e., steady-state-stacking in the presence of spacer ions (Ampholine) has been tested as a possible method for preparative scale fractionation of proteins on a very high load level. Optimal conditions for isotachophoresis have been worked out. However, it appears that the ampholine

spacer ions do not provide a satisfactory range of constituent mobilities, to permit the high resolution analysis of multi-component systems, such as human serum.

Measurements of the oxidation-reduction potential of polyacrylamide gels have been made. This is important, since it has been claimed that the persulfate, utilized in the polymerization of gels, can result in artifact bands. Methods to introduce reducing conditions in the gel have been developed.

Optimization of the conditions for preparative polyacrylamide gel electrophoresis: Optimization with regard to gel volume, pore size, elution buffer flow rate as a function of the K_R and Y_Q values of the species to be separated has been theoretically computed and experimentally validated.

Other Collaborative Studies:

1. Characterization of acid mucopolysaccharides;
2. Characterization and purification of mouse α1-antitrypsin;
3. Characterization of fetal, embryonic, and adult rabbit hemoglobins;
4. Characterization of glucagon, glucagon derivatives, and angiotensin.

II. Mathematical Biology

Theory of radioimmunoassays

Study of kinetics of separation of bound and free: A series of mathematical models have been developed to describe the effects of procedures used to separate bound and free forms of labeled antigen (such as charcoal adsorption, solid-phase methods, etc.) on the behavior of radioimmunoassay systems. Computer simulation studies have been performed. The methods utilized for the separation of bound and free antigen can have a marked effect on the shape of the dose response curve, on the shape of the Scatchard Plot, and on the apparent affinity constant and binding capacity derived from the Scatchard Plot. Experimental studies generally confirmed the predictions of the theory.

Study of immunoradiometric assays (labeled antibody assays): The behavior of immunoradiometric assay systems, which employ labeled antibody, has been studied by a series of mathematical models and computer simulation studies. This has elucidated a number of properties of the system, such as the effects of affinity constants, antibody concentration, specific activity, reaction volume, counting time, amount of immunoadsorbent, ratio of immunoadsorbent to antibody, etc. The theoretical sensitivity of immunoradiometric assay systems is very similar to that of radioimmunoassays. The factors governing the choice of labeled antigen, vis-a-vis labeled antibody systems were investigated. The experimental data appear to be in satisfactory accord with the theoretical predictions.

Development of parameter-fitting programs for any number of antigens reacting with any number of antibodies: This work makes it possible to

preparation is homogeneous, using polyacrylamide gel electrophoresis. Most of these are simple adaptations of standard statistical techniques such as the paired and unpaired Student's t test and analysis of variance. One new method has been developed for the construction of joint 95% confidence limits for the retardation coefficient (K_R) and the Y-intercept (Y_o). These methods have been fully computerized permitting routine use.

Theory of macromolecular mapping: Methods for the "finger-printing" of complex multicomponent systems have been developed. The theory underlying these methods has been developed and utilized for computer simulation. This has led to the development of several new techniques, involving transverse gel gradients in a two-dimensional slab apparatus. These methods will be tested during the coming year as new equipment becomes available for this purpose.

Fick's equation for electrophoretic transport on a preparative-scale gel electrophoresis column has been utilized as a basis for computer simulation. This, in turn, permits optimization of the length of the gel, gel concentration, ionic strength, voltage gradient, and other factors contributing to the performance of prep-columns. The predictions of this theory are being tested with studies on model proteins such as bovine serum albumin and hemoglobin and will be applied to the preparation of human prolactin.

III Tissue Culture

Choriocarcinoma cells: The major accomplishment has been the formation of hybrid somatic cell lines by fusing the JEG-3 line of choriocarcinoma cells with both human and mouse parent lines. Several of the hybrid lines showed synthesis of chorionic gonadotropin although production of progesterone and estradiol was extinguished. This represents the first demonstration of hormone synthesis in a hybrid somatic cell and indicates that repression of specialized or "luxury" function is specific and selective.

Cellular antigenicity of trophoblast: The antigenicity of trophoblast cells from culture has been studied using lymphocyte cultures. In comparison with many other tumors, trophoblast cells are hypoactive. This finding is a starting point for studies bearing on the immunologic non-reactivity of the placenta.

Effects of nisin: Nisin, an antibiotic containing αB-unsaturated amino acids has caused fetal absorption in the rat. It stops secretion of gonadotropin by trophoblast cells in culture at doses that have no apparent toxicity.

Porcine granulosa cells: A major accomplishment with this system has been the first demonstration that estrogens are potent stimulators of progesterone synthesis by granulosa cells in culture. This induction is blocked by cycloheximide indicating that protein synthesis is probably necessary for the effect. These results confirm studies in vivo which demonstrated that estrogen supported the corpus luteum. This system provides a convenient, simple system for investigating the hormonal requirements for progesterone synthesis by granulosa cells.

IV Protein and Polypeptide Hormones

Production of antisera: The accomplishments during the past year have resulted for the most part from application of a new method for producing antisera to hormones. We have shown that primary immunization with a few micrograms of either a protein or polypeptide hormone or, alternately, of a steroid hormone conjugated to a protein carrier, will regularly stimulate formation of specific antisera in high titers. Using this method, specific antisera have been produced in response to the alpha and beta subunits of human chorionic gonadotropin, human luteinizing hormone, to human follicle stimulating hormone, to estradiol, cortisol, corticosterone, testosterone, dehydroepiandrosterone and cyclic AMP, among others. These antisera have been used to develop radioimmunoassays and to perform physiologic studies on effects of hormone neutralization in vivo. In addition to studies done in our laboratories, the antisera have been given to investigators in the United States and Europe, accelerating investigations of structure-function relationships of gonadotropins in vivo and in vitro.

Gonadotropin subunits: Antisera to the beta subunits of human glycoproteins are usually specific for the intact hormone from which the subunit was derived. Thus, assays have been developed which specifically measure hCG in the presence of hLH and vice versa. These assays have been shown to detect hCG as early as the 5th post-ovulatory day of the menstrual cycle in which a woman conceives; to discern ectopic production of an hCG-like hormone by a series of human neoplasms not previously known to have the capacity for hCG synthesis; and to be useful both for diagnosis and for following response to treatment in women with trophoblastic neoplasms.

The subunits of hCG are intrinsically inactive biologically, previously observed biologic activity of these substances being due to contamination with minute amounts of native hormone.

The subunits of hCG failed to be concentrated in ovaries and were rapidly cleared from plasma of immature female rats.

Antigenic similarity and cross-reactivity among human glycoprotein hormones appeared to result from common antigenic determinants in the alpha subunits of these substances.

Antisera to human LH subunits have been produced. Using those sera and LH subunits from human ovine, rat and bovine sources, it was shown that between species immunologic cross reactivity resides in the hormone specific β subunit, whereas within species cross reactivity resides in the α subunit which appears to be interchangeable among all glycoprotein hormones, both human and non-human.

The immunologic and biologic activities of recombined hCG and bovine LH subunits have been characterized. Although considerable biologic activity is observed when hCGα and bovine LHβ are recombined (also conversely), immunologic activities do not differ significantly whether the hCG subunits

are assayed alone or combined with a complementary bovine LH subunit. Those observations imply that the appropriate configuration is attained on recombination for biologic activity and that the hybrid molecule is immunologically distinct from hCG.

The metabolism of hCG subunits labeled with ^{3}H was studied. The subunits are cleared rapidly from plasma and are not concentrated by the ovary. This is consistent with their lack of biologic activity in ventral prostate weight assays.

Prolactin: A heterologous, specific, sensitive assay for human prolactin was developed which utilized radioiodinated porcine prolactin trace and anti-porcine prolactin antibody which was selected on the basis of cross reactivity with a human plasma sample containing large quantities of biologically active prolactin. This assay, sufficiently sensitive to measure human prolactin in plasma from normal subjects, is being utilized in a number of physiological studies, including:

1. The effects of L-dopa, arginine and insulin on prolactin secretion in normal subjects and patients with hypothalamic or pituitary diseases;

2. Prolactin secretion during the menstrual cycle;

3. Prolactin secretion in patients with involuntary infertility; and

4. Effects of estrogens on prolactin secretion in normal females and males.

Differences in cross reactivity have been noted between plasma prolactin of patients with hypothalamic disease and patients with prolactin producing pituitary tumors. This suggests the possibility that human prolactin produced by tumors and in various disease states differ immunochemically.

Thyrotropin: The relationships between specific antisera to the α and β subunits of hCG and the biological activity of human TSH in the McKenzie assay were studied. Antisera to the α subunit of hCG inhibit the biologic activity of TSH. Interestingly, antisera to the β subunit of hCG also partially inhibit the biological activity of human TSH. These studies indicate immunologic similarity between antigenic determinants of the α subunits of TSH and hCG and also probably the β subunits of hCG and TSH.

LHRH: In order to study LHRH, a method developed in this laboratory for gentle iodination of proteins was adapted for iodination of LHRH. It was shown that the releasing hormone was monoiodinated and retained full biologic activity. A superfusion system to measure the release of LH and FSH from isolated rat pituitary glands has been introduced. The release of LH in response to LHRH is accompanied by cAMP release. Synthetic peptides related to LHRH are being characterized in this system for biologic activity.

Interaction of glycoprotein hormones with agarose-concanavalin A: The ability of agarose-coupled concanavalin A to bind carbohydrate has been applied to the chromatography of glycoprotein hormones. Concanavalin A binds the α-mannosyl residues of these hormones, which can then be eluted with α-methyl-D-glucopyranoside. Desialylated forms of hCG showed increased binding activity indicating that interaction is favored by the removal of intervening terminal saccharide residues. Applications of this insoluble protein-glycoprotein interaction have included:

1. Methods for fractionation of mixtures containing a range of intact and variably sialylated hCG molecules;

2. Purification of radioiodinated and other labeled hormones from reaction mixtures with improved binding characteristics for biological studies and hormone binding;

3. Studies on the fractionation of hCG of human pregnancy plasma and urine have shown that the elution profile of pregnancy plasma is more like the intact hormone and that of urine is similar to desialylated hormone. Monkey CG has also been extracted effectively from pregnancy blood. The elution pattern shows at least two different species of MCG;

4. Chromatography of antibody-bound HCG showed that this complex can be adsorbed to agarose-concanavalin A, confirming the lack of involvement of carbohydrate residues in antigen binding and also indicating that immune complex formation leaves the relevant carbohydrate groups free to react with concanavalin A.

A particularly useful application of this chromatographic procedure may lie in the analysis of glycoprotein hormones in small samples of plasma or urine or in the early stages of bulk fractionation of crude tissue or urinary extracts.

V Steroids

Assays: The improvement of the technique for producing high titer antisera facilitated development of a series of radioimmunoassays. Using high titer antisera, radioimmunoassays for the following steroids have been developed: dehydroepiandrosterone, cortisol, corticosterone, androstenediol, 17-hydroxypregnenolone, substance S, estradiol. The antisera are useful as experimental tools; the assays are essential for animal and clinical studies.

Dehydroepiandrosterone (DHA) and its sulfate are quantitatively the most important adrenal secretory products in man. Using the radioimmunoassay it was shown for the first time that DHA is usually secreted in higher amounts than its sulfate and that the gonads secrete only DHA.

VI Mechanism of Action

Binding of Gonadotropins: Gonadotropin binding sites of the rat testis

with high affinity for hCG and LH have been demonstrated and applied to the development of a radioligand-receptor assay system for hCG and LH of various species. The system has also been used to study structural effects on gonadotropin binding, showing that terminal sialic acid residues of LH and hCG are not necessary for uptake by tissue receptors, and that the subunits of these hormones are virtually devoid of intrinsic binding activity in vitro. The specific gonadotropin binding sites have extremely high affinity ($2.30 \times 10^{10} M^{-1}$) and low capacity ($10^{-12} M/g$) testis for hCG, and are associated with membrane fragments on differential centrifugation and electron microscopy; therefore, these sites have been provisionally regarded as LH/hCG receptors. Functional coupling of hormone binding to the adenylate cyclase system is not demonstrable in testis homogenates but is readily apparent in the intact testis.

Response to Gonadotropins

Structure-function relationships: The response of the intact testis to gonadotropin in vitro has been defined, employing radioimmunoassay of testosterone to measure androgen release in vitro. In contrast to dissected or sliced preparations, the intact rat testis has been shown to be highly sensitive to LH and hCG, with rises in testosterone production during incubation with physiological levels of these hormones. Desialylation of the gonadotropins causes only a moderate fall in steroidogenesis potency (to about 50%) in vitro, and removal of galactose further reduces activity to 20% of the normal value. Thus, dissociation of binding and steroidogenesis is apparent in the case of the modified hCG preparations and has been further shown to apply to binding and cyclic AMP production in vitro. Subunits of LH and hCG have also been evaluated for in vitro biologic activity and found to be devoid of intrinsic activity -- their apparent activities being entirely attributable to contamination with intact hormones.

Effects of binding on gonadotropins: The intact testis has also been used to examine the biological properties of hormones which have been dissociated from binding sites after prior uptake by the testis in vitro. Such "used" hormones completely retain the ability to bind to the testis in vitro and to stimulate cyclic AMP and testosterone production in the intact testis; in fact, those activities are enhanced due to selective purification of the tracer hormones during recovery from testis binding sites, an example of affinity chromatography on receptor sites.

cAMP release: Radioimmunoassay of cyclic AMP by a modification of the method of Stein has been established and used for correlative studies on hormone binding and testosterone production. Extensive release of cAMP into the medium during hCG stimulation in vitro has been found, the rise in cAMP production preceding the commencement of testosterone synthesis. The extreme sensitivity of the testis to hCG is again apparent, with detectable rise in cAMP production in response to as little as 1 ng/ml of hCG. The dose and time relations between hCG and cAMP formation have been defined, and the presence of "spare" hormone receptors in the testis has been established on the basis of ^{125}I-hCG binding studies and simultaneous estimation of cAMP and testosterone.

VII Ovarian Function and Related Areas

Experimental: It has been shown that estradiol stimulates early neonatal ovarian growth in rats. Estrogens enhance FSH binding either secondary to the induced granulosa cell proliferation or development of new binding sites in the granulosa cell. A derivative of these studies was the finding that the augmentation reaction which forms the basis for the bioassay of FSH is due to estrogen secretion induced by hCG. The high titer estradiol antiserum was most useful in proving these points. These data are necessary to define those factors that initiate follicular growth.

Clinical: Steroid secretion

The ovary was shown to secrete relatively small amounts of Δ^5-3β-hydroxy-steroids and there was no correlation with disease states. During stimulation with human menopausal gonadotropin, there was a marked increase in secretion of these steroids indicating disproportionate stimulation of the "Δ^5" pathway of steroid biosynthesis. The concurrent administration of dexamethasone delayed steroid secretion demonstrating for the first time that glucocorticoid administration has effects other than suppression of adrenal cortical function.

Amenorrheic states: A systematic study of women with secondary amenorrhea has been pursued. The response to clomiphene citrate and to progesterone define particular sub-groups of the population. These defined characteristics are being compared with the response to exogenous gonadotropins used for ovulation induction.

Pregnancy: The first study of the plasma concentrations of estradiol, estrone, estriol, and estrone sulfate throughout pregnancy was completed. Estriol was present in lowest concentration. The estrone sulfate: estrone and estradiol ratio was the same as in normal women showing that fetal estrone sulfate secretion was quantitatively unimportant. The plasma concentrations of all steroids correlated well with estimated fetal weight rather than with placental weight, emphasizing the known role of the fetus in the maintenance of maternal estrogen levels.

Inhibition of luteal function: Three normal volunteers received aminoglutethemide, an inhibitor of steroid synthesis, in an attempt to block progesterone synthesis by the corpus luteum. At tolerable doses, no effect was seen. Three normal volunteers were given estriol, an anti-estrogen under certain circumstances, in an attempt to decrease corpus luteum life span and function. At 20 mg daily, no effect was seen.

Uterine endometrium: Investigation into the steroidal control of carbonic anhydrase isoenzymes in uterine endometrium was extended to human subjects. The total carbonic anhydrase activity of human endometrium doubled during the secretory phase of the menstrual cycle. Erythrocytes contained two isoenzymes that were found also in both proliferative and secretory phase endometrium although these carbonic anhydrases were unaffected by hormonal changes that accompanied the normal menstrual cycle. In contrast, secretory phase endometrium contained a uterine-specific isoenzyme that was present only during

the luteal phase of the menstrual cycle.

VIII The Testis -- Clinical

Male Infertility Clinic

Over 100 patients have been seen in this clinic during the year, requiring about 1,000 patient visits for examinations, evaluation and treatment. This patient population provided case material for study of hypogonadotropism, Klinefelter's Syndrome, varicocele, cryptorchidism, germinal cell aplasia and a wide variety of oligospermic states. The spectrum of diseases and the facilities to evaluate these problems probably surpass those of any male infertility center in the world.

Testicular-Pituitary Interaction

FSH: Studies in men with a wide variety of testicular disorders support the previous hypothesis that tubular factors are important for FSH regulation. FSH levels are elevated in approximately half of the men studied while LH and testosterone are essentially within the normal range. FSH titers do not correlate with sperm concentration. There appears to be a trend in the data that FSH levels are more generally elevated when the tubular disease is more severe. The complete loss of germ cells is uniformly associated with elevated FSH.

Steroids: The increase in FSH level is not associated with a concomitant decrease in sex steroid level. The basal concentrations of testosterone, estradiol, 17-hydroxyprogesterone and androstenediol are not different from those of normal men; furthermore, the increase in steroid concentrations following four days of hCG stimulation is identical in normal and infertile men. Therefore, it is difficult to attribute the selective increase in FSH in infertile men to any abnormality in Leydig cell function.

Effects of steroids on gonadotropins: To study this interaction, 20 men were infused for 4 days with physiological amounts of testosterone, estradiol, estradiol plus 17-hydroxyprogesterone or dihydrotestosterone. Testosterone produced proportional decrease in FSH and LH to approximately 1/3 of the control level. Estradiol produced identical changes. Thus, estradiol may well be the effective Leydig cell inhibitor of FSH secretion since peripheral conversion of testosterone accounts for the majority of estradiol in men. Estradiol plus 17-hydroxyprogesterone produced somewhat greater decreases in FSH and LH levels than estradiol alone. However, dihydrotestosterone produced only minimal decreases in LH concentrations and no change in FSH levels. The above data strengthen the concept of a dual control mechanism for FSH concentration.

The threshold for FSH suppression by estrogen has been determined in normal men. A differential set-point for FSH and LH was determined.

Experimental

A study of the growth and development of the testis in the rat is oriented toward establishing parameters which mark the action of FSH in its initiation of spermatogenesis. Histochemical and biochemical methods were set up to assess several enzymes known to be important testicular components: Lactate Dehydrogenase (LDH), Sorbitol Dehydrogenase, γ-Glutamyl transpeptides. Unfortunately, enzymes as markers of spermatogenesis do not, at present, provide any additional information regarding spermatogenesis other than that determined by careful histological evaluation. However, the temporal appearance of these enzymes clearly reflect maturation of the tubule and appearance of germ cells.

The testis of the mouse has long been known to contain specific isoenzymes of LDH which appear when sperm are present. These LDH isoenzymes were found to be charge isomers rather than molecules of widely varying molecular size. Three new testicular isozymes related to the appearance of germ cells were noted. These isozymes are also seen in the ejaculated sperm. They are clearly different from the isozymes of immature testicular tissue, heart or muscle.

IX Other Clinical Studies

Hand-Schuller-Christian Disease

A thorough evaluation of pituitary function in a group of 13 patients with Hand-Schuller-Christian Disease has been made. The results of provocative tests for growth hormone indicate a good correlation between an inadequate growth hormone response and growth retardation. This lack of growth hormone release is probably the result of hypothalamic lesions since the anterior pituitary is usually spared from invasion by abnormal cells. Many of these patients have received radiotherapy to the hypothalamus to attempt to reverse the growth hormone defect. Long-term follow-up studies are necessary for evaluation.

Cushing's Syndrome

Two patients referred to the NIH because of severe osteoporosis were shown to have Cushing's Syndrome due to micronodular hyperplasia. The clinical manifestations of the disease were mild and the diagnosis was proved by the measurement of urinary free cortisol, using the newly-developed radioimmunoassay for cortisol. This rare syndrome can easily be missed clinically and it may be a more common cause of "ioiopathic" osteoporosis than has been realized.

Virilizing States

From the study of a patient with adolescent virilization, it is clear that previous dogma about the presence of a testis and fetal virilization is not always true. This patient had normal external genitalia, developed virilization at age 13, had adult male plasma concentrations of testosterone

and cryptorchid testes. This finding introduces new considerations into the appraisal of Leydig cell function during fetal life.

Significance to Bio-Medical Research and to the Institute:

The single largest area of research progress concerns the biochemistry and physiology of the gonadotropins. Since these protein hormones regulate ovarian and testicular steroid secretion, follicular maturation, and spermatogenesis, the acquisition of basic knowledge here is of great importance.

Members of the Branch have developed methods for studying and subsequently defining structural features of the gonadotropins necessary for function. The process of binding of the gonadotropin to the cell and the consequent initiation of a series of intracellular events are being defined and constitute critical steps for the possibility of manipulating gonadotropin action.

Derivative from these studies are data relating to the process of follicular maturation, regulation of spermatogenesis, and control of gonadal secretions. Investigations of fertility and sterility must begin at the biologic level in areas such as these.

Proposed Course:

Mechanism of action of gonadotropins will remain an area of intense investigation. Attempts will be made to define the hormone receptor interaction. Of particular importance will be the initiation of studies of spermatogenesis and the role of the gonadotropins.

Two relatively new areas of research, follicular maturation and releasing hormones will be expanded. The events associated with the progression of the primordial follicle to the graafian follicle will be defined. Biochemical studies with LHRH have been initiated and will receive increasingly greater support during the coming years.

Honors and Awards:

Mortimer B. Lipsett

Invited Lectures

American College of Physicians, Duke University, September 1971
Pan-American Endocrine Congress, Caracas, October 1971
Endocrine Society, Post-graduate Course, San Francisco, October 1971
Sloan-Kettering Institute, New York, October 1971
Jewish Hospital Medical Center, New York, October 1971
Pacific Coast Fertility Society, Palm Springs, November 1971
American Cancer Society, Florida, January 1972
American College of Physicians, Boston, March 1972
Massachusetts Institute of Technology, Boston, April 1972
Howard University, Washington, D. C., April 1972

Scientific Consultant, Veterans Administration, Washington, D. C.
Adviser and lecturer - National Heart and Lung Institute Symposium on Hormones and Atherosclerosis
Scientific Consultant, Agency for International Development, Washington, D C.

Visiting Professor

University of Iowa School of Medicine, Iowa City, November 1971
Washington University School of Medicine, St. Louis, May 1972
Vanderbilt University School of Medicine, Nashville, May 1972
Indiana University School of Medicine, Indianapolis, May 1972

G. T. Ross

Invited lectures

Newark Beth Israel Medical Center, Rita S. Finkler Lecture, October 1971
American Fertility Society, February 1972
American College of Physicians Post-graduate course, Boston University Medical Center, March 1972
Mayo Clinic, Division of Endocrinology, Rochester, Minnesota, April 1972
Minnesota OB-GYN Society, Rochester, Minnesota, April 1972
University of Minnesota, Minneapolis, Minnesota, April 1972
University of Texas Medical Branch, Galveston, Texas, May 1972
Serono Research Foundation, Boston, May 1972
IV International Congress of Endocrinology, Washington, D. C., June 1972

Wm. W. Tullner

Invited lecture

World Health Organization Symposium on the Use of Non-Human Primates for Research on Problems of Human Reproduction. Sukhumi, USSR, December 1971

Andreas Chrambach

Invited lectures

Session Chairman and lecturer at International Conference on Isoelectric Focusing and Isotachophoresis, New York Academy of Sciences, May 1972
Panel Speaker, Gordon Conference on Interactions of Proteins with Small Molecules, New Hampton, N. H., June 1972

Peter O. Kohler

Invited lectures

University of Texas at San Antonio, November 1971
Baylor Medical School, Houston, Texas, November 1971

Wm. E. Bridson

Invited lectures

Endocrinology Research Seminar, Department of Medicine, University of Wisconsin, October 1971
Medical Grand Rounds, University of Wisconsin, October 1971
Tenovus Institute Participant - IVth Tenovus Work Shop on Prolactin and Carcogenesis, Welsh National School of Medicine, Cardiff, Wales, March 1972

K. J. Catt

Invited lectures

I.N.S.E.R.M.-CEA-Symposium on Radioimmunoassay, Paris, May 1972
Visiting Professor-University of West Virginia Medical Center, Morgantown, West Virginia, October 1971

M. L. Dufau Catt

Invited lecture

I.N.S.E.R.M.-CEA-Symposium on Radioimmunoassay, Paris, May 1972

Richard J. Sherins

Invited lectures

American Fertility Society, Post-graduate course, New York, February 1972
Symposium, Hormonal Control of Spermatogenesis-Washington, D. C. June 1972

David Rodbard

Invited lectures

Symposium on Interaction of Steroids with Macromolecules, European Journal of Steroid Biochemistry, Paris, September 1971
Radioimmunoassay Data Processing, UCLA Medical Center, Los Angeles, January 1972
New York Academy of Sciences, Conference on Isoelectric Focusing, New York May 1972
Theory of Radioimmunoassays, New Developments, IV International Congress of Endocrinology, Washington, D. C., June 1972
Introduction to Radioimmunoassays and Immunoradiometric Assays, Chemistry Department, Georgetown University, Washington, D. C., February 1972

Howard Kulin

Invited lectures

George Washington University School of Medicine, Washington, D. C., April 1972
College of Physicians & Surgeons, Columbia University, New York, April 1972

Yale University School of Medicine, Department of OB-GYN, New Haven, May 1972

Judith L. Vaitukaitis

Invited lectures

College of Physicians and Surgeons, Columbia University, New York, December 1971
University of Michigan School of Medicine, Ann Arbor, Michigan, January 1972
III Pan American Congress of Anatomy, New Orleans, March 1972
Boston University School of Medicine, Boston, May 1972

Publications

Protein Chemistry

Chrambach, A. and Rodbard, D.: Polyacrylamide gel electrophoresis. Science 172:440-451, 1971

Corvol, P. L., Chrambach, A., Rodbard, D., and Bardin, C. W.: Physical properties and binding capacity of testosterone-estradiol-binding globulin in human plasma, determined by polyacrylamide gel electrophoresis. J. Biol. Chem. 246:3435-3443, 1971

Dante, M. L. and Chrambach, A.: Multiphasic buffer systems: Retrieval program for systems output, National Technical Information Service, Springfield, Virginia, PB 203016, 1971

Dante, M. L. and Chrambach, A.: Buffers and PAGE. Science 175:95, 1972

Doerr, P. and Chrambach, A.: Anti-estradiol antibodies: Isoelectric focusing in polyacrylamide gel. Anal. Biochem. 42:96-107, 1971

Kapadia, G. and Chrambach, A.: Recovery of protein in preparative polyacrylamide gel electrophoresis. Anal. Biochem. In Press

Kaplan, G. N., Maffezzoli, R. D., and Chrambach, A.: Characterization of human chorionic gonadotropin and luteinizing hormone contained in urinary extracts by polyacrylamide gel electrophoresis. J. Clin. Endocr. 34:370-374, 1972

Maffezzoli, R. D., Kaplan, G. N., and Chrambach, A.: Fractionation of immunoreactive human chorionic gonadotropin and luteinizing hormone by isoelectric focusing in polyacrylamide gel. J. Clin. Endocr. 34:361-369, 1972

Maffezzoli, R. D., Kaplan, G. N., and Chrambach, A.: Physical characterization by polyacrylamide gel electrophoresis of human follicle stimulating hormone from urinary extract of children, men and pre- and post-menopausal women. J. Clin. Endocr. 34:375-379, 1972

Miles, L. E. M., Simmons, J. E., and Chrambach, A.: Instability of pH gradients in isoelectric focusing on polyacrylamide gel. Anal Biochem. In Press

Myerowitz, R. L., Chrambach, A., Rodbard, D., and Robbins, J. B.: Isolation and characterization of mouse serum alpha 1-antitrypsins. Anal. Biochem. In Press

Ugel, A. R., Chrambach, A., and Rodbard, D.: Fractionation and characterization of an oligomeric series of bovine keratohyalin by polyacrylamide gel electrophoresis. Anal. Biochem. 43:410-426, 1971

Mathematical Biology

Feldman, H., Rodbard, D., and Levine, D.: Mathematical theory of cross-reactive radioimmunoassay and ligand-binding systems at equilibrium. Anal. Biochem. 45:530-556, 1972

Rodbard, D. and Catt, K. J.: Mathematical theory of radioligand assays: the kinetics of separation of bound from free. J. Ster. Biochem. In Press

Rodbard, D., Ruder, H. J., Vaitukaitis, J. L., and Jacobs, H. S.: Mathematical analysis of kinetics of radioligand assays: improved sensitivity obtained by delayed addition of labeled ligand. J. Clin. Endocr. 33:343-355, 1971

Tissue Culture

Bridson, W. E., Ross, G. T., and Kohler, P. O.: Immunologic and biologic activity of chorionic gonadotropin synthesized by cloned choriocarcinoma cells in culture. J. Clin. Endocr. 33:145-149, 1971

Hammond, J. M., Bridson, W. E., Chrambach, A., and Kohler, P. O.: Isohormones of chorionic gonadotropin produced by cloned cell lines in culture. J. Clin. Endocr. 34:185-187, 1972

Hammond, J. M., Bridson, W. E., Kohler, P. O. and Chrambach, A.: Physical characteristics of immunoreactive chorionic gonadotropin produced in culture. Endocrinology 89:801-806, 1971

Kohler, P. O. and Bridson, W. E.: Isolation of hormone-producing clonal lines of human choriocarcinoma. J. Clin. Endocr. 32:683-687, 1971

Kohler, P. O., Bridson, W. E., Hammond, J. M., Weintraub, B., Kirschner, M. A., and Van Thiel, D. H.: Clonal lines of human choriocarcinoma cells in culture. Acta. Endocr. Supp. 153:137-153, 1971

Van Thiel, D. H., Bridson, W. E., and Kohler, P. O.: Induction of luteinization in cultures of granulosa cells by chorionic gonadotropins. Endocrinology 89:622-624, 1971

Protein and Polypeptide Hormones

Cain, M. D., Coghlan, J. P., and Catt, K. J.: Measurement of Angiotensin II in blood by radioimmunoassay. Clin. Chim. Acta. In Press

Cain, M. D., Walters, W. A., and Catt, K. J.: Effects of oral contraceptive therapy on the renin-angiotensin system. J. Clin. Endocr. 33:671-676, 1971

Catt, K. J., Dufau, M. L., and Tsuruhara, T.: Radioligand-receptor assay of luteinizing hormones and chorionic gonadotropins. J. Clin. Endocr. 34: 123-132, 1972

Chrambach, A., Bridson, W. E., and Turkington, R. W.: Human prolactin identification and physical characterization of the biologically active hormone by polyacrylamide gel electrophoresis. Biochem. Biophys. Res. Comm. 43:1296-1303, 1971

Coble, Y., Kohler, P. O., Tullner, W. W., and Cargille, C. M.: Clearance of iodinated human follicle stimulating hormone in Macaca Mulatta. J. Endocr. 51:783-784, 1971

Dufau, M. L., Catt, K. J., and Tsuruhara, T.: Retention of in vitro biological activities by desialylated human luteinizing hormone and chorionic gonadotropin. Biochem. Biophys. Res. Comm. 44:1022-1029, 1971

Kulin, H. E., Leiter, E. O., and Bridson, W. E.: Pubertal maturation of the gonadotropin stimulatory response to clomiphene: Case report. J. Clin. Endocr. 33:551-553, 1971

Menard, J. and Catt, K. J.: Measurement of renin activity, concentration and substrate in rat plasma by radioimmunoassay of Angiotensin I. Endocrinology 90:422-430, 1972

Miyachi, Y. and Chrambach, A.: Structural integrity of gonadotropins after enzymatic iodination. Biochem. Biophys. Res. Comm. 46:1213-1221, 1972

Miyachi, Y., Vaitukaitis, J. L., Nieschlag, E., and Lipsett, M. B.: Enzymatic radioiodination of gonadotropins. J. Clin. Endocr. 34:23-28, 1972

Nixon, W. E. and Cargille, C. M.: Binding of tracer FSH, LH, GH and TSH by anti-FSH sera prepared by several immunization regimens. J. Lab. Clin. Med. 78:949-956, 1971

Nixon, W. E., Hodgen, G. D., Niemann, W. H., Ross, G. T., and Tullner, W. W.: Urinary chorionic gonadotropin in middle and late pregnancy in the chimpanzee. Endocrinology 90:1105-1109, 1972

Rayford, P. L., Brinkley, H., and Young, E. P.: Radioimmunoassay determination of LH concentration in the serum of female pigs. Endocrinology 88:707-713 1971

Sato, N. and Cargille, C. M.: Separation of specific from non-specific anti-FSH antibody by affinity chromatography on sepharose-HCG. Endocrinology 90: 302-306, 1972

Vaitukaitis, J. L., Robbins, J. B., Nieschlag, E., and Ross, G. T.: A method for producing specific antisera in small doses of immunogen. J. Clin. Endocr. 33:1049-1052, 1971

Vaitukaitis, J. L. and Ross, G. T.: Altered biologic and immunologic activities of progressively desialylated human urinary FSH. J. Clin. Endocr. 33:308-311, 1971

Vaitukaitis, J. L., Sherins, R. J., Ross, G. T., Hickman, J., and Ashwell, G.: A method for the preparation of radioactive FSH with preservation of biologic activity. Endocrinology 89:1356-1360, 1971

Van Hall, E. V., Vaitukaitis, J. L., Ross, G. T., Hickman, J. W., and Ashwell, G.: Effects of progressive desialylation on the rate of immunoreactive HCG from plasma in rats. Endocrinology 89:11-15, 1971

Steroids

Bermudez, J. A., and Lipsett, M. B.: Early adrenal response to ACTH: Plasma concentrations of pregnenolone, 17-hydroxypregnenolone, progesterone, and 17-hydroxyprogesterone. J. Clin. Endocr. 34:241-243, 1972

Dufau, M. L., Catt, K. J., Tsuruhara, T., and Ryan, D.: Radioimmunoassay of plasma testosterone. Clin. Chim. Acta 37:109-116, 1972

Kirschner, M. A. and Jacobs, J. B.: Combined ovarian and adrenal vein catheterization to determine the site(s) of androgen overproduction in hirsute women. J. Clin. Endocr. 33:199-209, 1971

Loriaux, D. L., Ruder, H. J., and Lipsett, M. B.: The measurement of estrone sulfate in plasma. Steroids 18:463-472, 1971

Loriaux, D. L., Lipsett, M. B.: Radioligand assay for Δ^5-3β-hydroxysteroids II. 5-Androstene-3β, 17β-diol and 3β, 17α-dihydroxy-5-pregnen-20-one. Steroids, In Press

Mahoudeau, J. A., Bardin, C. W., and Lipsett, M. B.: The metabolic clearance rate and origin of plasma dihydrotestosterone in man and its conversion to the 5 androstanediols. J. Clin. Invest. 50:1338-1344, 1971

Nieschlag, E. and Loriaux, D. L.: Radioimmunoassay for plasma testosterone. Z. klin. Chem. u. klin. Biochem. 10:164-168, 1972

Nieschlag, E., Loriaux, D. L., and Lipsett, M. B.: Radioligand Assay for Δ^5-3β-hydroxysteroids I. 3β-hydroxy-5-androstene-17 one and its 3 sulfate. Steroids, In Press

Parks, G. A., Bermudez, J. A., Anast, C. A., Bongiovanni, A. M., and New, M.I.: Pubertal Boy with 3β-hydroxy steroid dehydrogenase defect. J. Clin. Endocr. 33:269-278, 1971

Ruder, H. J., Corvol, P., Mahoudeau, J. A., Ross, G. T., and Lipsett, M. B.: Effects of induced hyperthyroidism on steroid metabolism in man. J. Clin. Endocr. 33:382-387, 1971

Ruder, H. J., Loriaux, D. L., and Lipsett, M. B.: Estrone sulfate: Production rate and metabolism in man. J. Clin. Invest. 51:1020-1033, 1972

Wright, L. J., Lipsett, M. B., Ross, G. T., and Wolff, S. M.: Effects of dexamethasone and aspirin on the responses to endotoxin in man. J. Clin. Endocr. 34:13-17, 1972

Mechanism of Action

Bullock, L. P., Bardin, C. W., Gram, T. E., Schroeder, D. H., and Gillette, J. R.: Hepatic ethylmorphine demethylase and Δ^4-steroid reductase in the androgen-insensitive pseudohermaphroditic rat. Endocrinology 88:1520-1523, 1971

Catt, K. J., Dufau, M. L., and Tsuruhara, T.: Studies on a radioligand receptor assay system for luteinizing hormone and chorionic gonadotropin. J. Clin. Endocr. 32: 860-863, 1971

Dufau, M. L., Catt, K. J., Tsuruhara, T.: Gonadotropin stimulation of testosterone production by the rat testis in vitro. Biochim. Biophys. Acta 252:574-579, 1971

Dufau, M. L., Catt, K. J., and Tsuruhara, T.: A sensitive gonadotropic response system: Radioimmunoassay of testosterone production by the rat testis in vitro. Endocrinology 90:178-186, 1972

Schneider, G., French, A., Bullock, L. P., and Bardin, C. W.: Absence of androgen stimulation of hepatic steroid sulfatase in the female rat. Endocrinology 89:308-310, 1971

Sherins, R. J. and Bardin, C. W.: Preputial gland growth and protein synthesis in the androgen-insensitive male pseudohermaphroditic rat. Endocrinology 89: 835-841, 1971

Sherins, R. J., Bullock, L. P., Gay, V. I., Vanha-Perttula, T., and Bardin, C. W.: Plasma LH and FSH levels in the androgen insensitive pseudohermaphroditic rat: responses to steroid administration. Endocrinology 88:763-770, 1971

Vanha-Perttula, T., Schneider, G., Bardin, C. W., Stanley, A. J., Gumbreck, L. G., and Allison, J. E.: Testicular tumors in the androgen insensitive pseudohermaphrodite rat: Histochemistry and steroid metabolism. Gynec. Invest. 1:149-160, 1970

Ovarian Function and Related Areas

Falk, R. J. and Hodgen, G. D.: Carbonic anhydrase isoenzymes in normal human endometrium and erythrocytes. Amer. J. Obstet. Gynec. In Press

Hodgen, G. D. and Falk, R. J.: Estrogen and progesterone regulation of carbonic anhydrase isoenzymes in guinea pig and rabbit uterus. Endocrinology 89:859-864, 1971

Hodgen, G. D. and Falk, R. J.: A radiotracer assay for carbonic anhydrase. Intl. J. Appl. Radiat. Isotopes 22:492-495, 1971

Shimkin, P. M., Van Thiel, D. H., and Ross, G. T.: Selective hypogastric arteriography in uterine choriocarcinoma. Amer. J. Roentgen. CXI:535-540, 1971

Slivka, J., Cargille, C. M., and Ross, G. T.: An analysis for superimposed biological rhythms: application to FSH and LH in women. J. Appl. Physiol. 30:905-908, 1971

Van Thiel, D. H., Grodin, J. M., Ross, G. T., and Lipsett, M. B.: Partial placenta accreta in pregnancies following chemotherapy for gestational trophoblastic neoplasms. Amer. J. Obstet. Gynec. 112:54-58, 1972

The Testis

Coble, Y., Bardin, C. W., Ross, G. T., and Darby, W. T.: Studies of endocrine function in boys with retarded growth, delayed sexual maturation and zinc deficiency. J. Clin. Endocr. 32:361-367, 1971

Hodgen, G. D.: Total glycerol in the excurrent ducts of the male rat. J. Reprod. Fert. 28:277-280, 1972

Hodgen, G. D. and Gawienowski, A. M.: Uptake in vitro of ^{14}C, 5-hydroxytryptamine and ^{14}C, hydroxytryptophan by reproductive tract tissues of the adult male rat. J. Reprod. Fert. 28:291-293, 1972

Hodgen, G. D., Gomes, W. R., and VanDemark, N. L.: A testicular isoenzyme of carbonic anhydrase. Biol. Reprod. 4:224-228, 1971

Gawienowski, A. M. and Hodgen, G. D.: Homosexual activity in male rats after p-chlorophenylalanine: effects of hypophysectomy and testosterone. Physiol. Behav. 7:551-555, 1971.

Reiter, E. O. and Kulin, H. E.: Suppressed follicle stimulating hormone in men with chorionic gonadotropin secreting testicular tumors. J. Clin. Endocr. 33:957-961, 1971

Other Clinical Studies

Berman, M. L., Hanson, K., and Hellman, I.: Effect of breast feeding on post partum menstruation, ovulation, and pregnancy in Alaskan Eskimos. Amer. J. Obstet. Gynec. In Press

Reviews and Chapters

Bardin, C. W., Bullock, L. P., Blackburn, W. R., Sherins, R. J., and Vanha-Perttula, T.: Testosterone metabolism in the androgen-insensitive rat: a model for testicular feminization. In McKusic, V. (ed.); Third Conference on the Clinical Delineation of Birth Defects, Vol. VII (Part 10). Baltimore, Johns Hopkins Medical Institutions and the National Foundation of the March of Dimes, 1971, pp. 185-192

Bardin, C. W. and Kirshner, M. A.: The clinical usefulness of testosterone measurements in virilizing syndromes in women. In Sunderman, F. W. and Sunderman, Jr., F. W. (Eds.): Laboratory Diagnosis of Endocrine Diseases. St. Louis, Warren H. Green, Inc., 1971, pp. 559-572

Cargille, C. M.: Immunologic methods for the measurement of gonadotropins in biologic fluids in man. In Sunderman, F. W. and Sunderman, Jr., F. W. (eds.): Laboratory Diagnosis of Endocrine Diseases, St. Louis, Warren H. Green, Inc. 1971, pp. 152-160

Cargille, C. M. and Rayford, P. L.: Double antibody radioimmunoassay for plasma gonadotropins. In Sunderman, F. W. and Sunderman, Jr., F. W. (eds.): Laboratory Diagnosis of Endocrine Diseases, St. Louis, Warren H. Green, Inc. 1971, pp. 161-175

Catt, K. J.: Radioimmunoassay studies of the renin-angiotensin system in human hypertension and during estrogen treatment. In Hypertension, Springer-Verlag, Germany, In Press

Feldman, H. and Rodbard, D.: Mathematical theory of radioimmunoassay. In Odell, W. and Daughaday, W. (eds.): Competitive Protein Binding Assay, Philadelphia, J. B. Lippincott Co., 1971, pp. 158-203

Kirschner, M. A.: The measurement of testosterone and adnrostenedione in biological fluids (electron-capture gas-liquid chromatography). In Sunderman, F. W. and Sunderman, Jr., F. W. (eds.): Laboratory Diagnosis of Endocrine Diseases, St. Louis, Warren H. Green, Inc. 1971, pp. 547-554

Lipsett, M. B.: Steroid secretion by the gonads in man. Excerpta Medica, Proc. of III Intl. Cong. on Hormonal Steroids, Hamburg, 1970, pp. 7-13

Lipsett, M. B.: Endocrine mechanisms of reproduction. In Frohlich, E. D. (ed.): Pathophysiology, Altered Regulatory Mechanisms in Disease, Philadelphia, J. B. Lippincott Co., 1972, pp. 355-382

Lipsett, M. B.: Clinical considerations of 17-ketosteroid and testosterone measurements. In Sunderman, F. W. and Sunderman, Jr., F. W. (eds.): Laboratory Diagnosis of Endocrine Diseases, St. Louis, Warren H. Green, Inc. 1971, pp. 555-558

Lipsett, M. B.: Factors influencing the rate of metabolism of steroid hormones in man. Ann. N. Y. Acad. Sci. 179:442-449, 1971

Lipsett, M. B.: Interconversions among steroid hormones and precursors. In Sunderman, F. W. and Sunderman, Jr., F. W. (eds.): Laboratory Diagnosis of Endocrine Diseases, St. Louis, Warren H. Green, Inc. 1971, pp. 84-88

Odell, W. D. and Ross, G. T.: Correlation of bioassay and immunoassay potencies for FSH, LH, TSH and HCG. In Odell, W. D. and Daughaday, W. H. (eds.): Principles of Competitive Protein Binding Assays, Philadelphia, J. B. Lippincott Co., 1971, pp. 401-418

Rodbard, D.: Statistical aspects of radioimmunoassay. In Odell, W. D. and Daughaday, W. H. (eds.): Principles of Competitive Protein Binding Assays, Philadelphia, J. B. Lippincott Co., 1971, pp. 204-259

Ross, G. T.: Biological methods for the measurement of gonadotropin in man. In Sunderman, F. W. and Sunderman, Jr., F. W. (eds.): Laboratory Diagnosis of Endocrine Diseases, St. Louis, Warren H. Green, Inc., 1971, pp. 138-147

Ross, G. T.: Factors governing choice of a reference preparation for competitive binding assays. In Odell, W. D. and Daughaday, W. H. (eds.): Principles of Competitive Protein Binding Assays, Philadelphia, J. B. Lippincott Co., 1971, pp. 325-338

Ross, G. T.: Pituitary-gonadal interactions prior to and after sexual maturity in man. J. Periodont. 42:468-470, 1971

Ross, G. T., Brice, J., and Reid, R.: Biologic methods for determination of urinary gonadotropin activity. In Sunderman, F. W. and Sunderman, Jr., F.W. (eds.): Laboratory Diagnosis of Endocrine Diseases, St. Louis, Warren H. Green, Inc. 1971, pp. 148-151

Ross, G. T., Vaitukaitis, J., and Robbins, J. B.: Preparation and properties of antisera made to alpha and beta subunits of human chorionic gonadotropin. In Margoulies, M. and Greenwood, C. (eds.): Protein and Polypeptide Hormones, Amsterdam, Excerpta Medica Foundation, 1971, pp. 153-157

Tullner, W. W.: Chorionic gonadotropin in non-human primates. In Diczfalusy, E. (ed.): Symposium on the use of non-human primates on problems of human reproduction, Sukhumi, USSR, 1971, In Press

Tullner, W. W.: Comparative aspects of primate chorionic gonadotropins. In Luckett, W. P., Noback, C., and Montagna, W. (eds.): Advances in primatology, Vol. 2, Reproductive Biology of the Primates, New York, Appleton-Century-Crofts, In Press

Tullner, W. W. and Hertz, R.: Comparative aspects of chorionic gonadotropin production in non-human primates. In Moor-Jankowski, J. and Goldsmith, E. (eds.): Medical Primatology, Proc. 2nd Conf. on Exper. Med. Basel, Karger, 1971, pp. 553-561

NICHD Annual Report
July 1, 1971 through June 30, 1972
Reproduction Research Branch
Reproduction Anthropometry Section

A Summary Statement

This Section provides a data base from which menstrual and reproduction characteristics of normal women may be defined. Through analyses of these data, the Section provides information in clarification of the basic biology of the human reproductive processes as they relate to different groups of women and a measure of relevance of clinical and laboratory findings.

Although data collection operations continue, all processing of the data for purposes of analyses was completed this year. The coded data are stored on computer tape in the Division of Health Computer Sciences (DHCS) of the University of Minnesota. The DHCS functions for this purpose under a contract with the National Institutes of Health, this Section providing direction on behalf of NICHD through provision of the Project Officer.

Available for analytical studies from DHCS are computer tapes for a Caucasian population which includes information for over 3,000 women covering approximately 40,000 person-years of genital bleeding, reproduction and pregnancy control; another for an Eskimo population including 1,293 women with almost 3,100 person-years experience; and a third for 200 women representing the Caucasian population in Alaska. In addition, information on age at menarche has been assembled for 801 girls in native schools in Alaska.

It was proposed as a result of laboratory studies that women with short menstrual cycles were more likely to be infertile than those with longer cycles. A test of this proposal was initiated during the year using the data from the population of women participating in the Menstrual and Reproduction History Program. Analysis of these data showed that the rate of conception did indeed increase with the average length of the menstrual cycle. In addition, more habitual aborters were found among women with the shortest cycles than among those with longer cycles with a complete absence of habitual aborters among women with cycles averaging thirty days or more. Occasional aborters, on the other hand, are found among women regardless of length or variability of menstrual cyclicity. So far as can be ascertained, such findings have not been previously reported from population data. Further analyses are in progress to differentiate the reproductive characteristics of women as these are associated with their menstrual characteristics.

Serial No. HD-RP4(7)(C)
1. Reproduction Research Branch
2. Reproduction Anthropometry Section
3. NIH, Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Rhythmometry of Genital Bleeding as a Function of Human Reproduction

Principal Investigator: Alan E. Treloar, Ph.D.

Other Investigator: Ida L. Hellman, M.Sc.

Man Years:

Total: 4.4
Professional: 2.0
Other: 2.4

Project Description:

Objectives:

1. To study the association of fertility status of women and their menstrual characteristics.
2. To compare the menstrual characteristics of women terminating in natural menopause with those terminating in hysterectomy during the ten years prior to termination.
3. To study the patterns of menstrual cyclicity in the early years after menarche among sisters.
4. To study the patterns of usage of oral contraceptive drugs.

Methods Employed:

In answer to questions asked, to solve existing problems found in literature reviews and to describe the population under study, pilot studies are conducted from the original records to indicate significant relationships between variables. From results of these preliminary analyses, tabular presentations are requested from DHCS, analyzed and the findings prepared for publication.

Major Findings:

A tabulation of fertility in the first ten years of marriage of 704 women not using contraceptive drugs or IUD's in relation to the average length of their menstrual cycle and its variability was analyzed. We found (1) the conception rate increases as average length of menstrual cycle

increases, and (2) habitual aborters are limited to women with short menstrual cycles, especially those with the shortest cycles and the least variability.

The pilot study of the comparison of the menstrual cyclicity of women terminating their reproductive life span through natural menopause with those undergoing hysterectomies indicates that for the ten years before termination spotting between menstrual periods and longer duration of flow separates women undergoing hysterectomy from those terminating with natural menopause. Computer tabulations have been requested for 373 women who have reached menopause and 201 women who have undergone hysterectomy.

A pilot study of age at menarche and the pattern of menstrual cyclicity in the early years after menarche among sisters gave no clear results. A tabular presentation of the data from the computer may make it easier to visualize these relationships.

Patterns of usage of oral contraceptive drugs are being studied from the original records of as many as 1000 women. The rate of usage by age and year shows that, in this population, a little over 50 per cent of the participants in the study under age 30 used the drugs to some extent during 1967, '68 and '69. During this same time period, between 10 and 11 per cent of women in the menopausal years also used the drugs. Analyses are continuing in order to show reasons for stopping usage, time to pregnancy when this is the reason for stopping, nature of disorders associated with usage, and the like. The use of drugs by the older population will be analyzed in relation to hysterectomy and menopause as these occur in this population of drug users and the impact of the drugs on the general pattern of menstrual termination evaluated.

Significance to Bio-medical Research and the Program of the Institute:

The findings from these and similar studies are basic to an understanding of the phenomena of reproductive biology as they occur in an unselected population. Thus they serve not only to substantiate the findings from clinical investigations conducted in the laboratory, but may open new avenues of approach to further studies in this area.

Proposed Course:

Further analyses will be conducted in each of these studies using computer tabulations whenever possible. It is expected that the findings will be submitted for publication.

Honors and Awards: Alan E. Treloar

Invited lecturer - 7th World Congress on Fertility and Sterility, Tokyo, October 1971

Conference on Natural Family Planning, Human Life Foundation, Airlie, Virginia, January 1972

NICHD Annual Report
July 1, 1971 through June 30, 1972

Reproduction Anthropometry Section
Collaborative Research Contract Report

Contract Title: Quantitative Definition of the Temporal Characteristics of the Human Menstrual Cycle

Contractor: University of Minnesota, Division of Health Computer Sciences

Amount Allocated: $45,375

Principal Investigator: Alan E. Treloar, Ph.D.

Contract Description:

Objectives:

To provide services in data processing and statistical analysis for studies of genital bleeding as a function of human reproduction.

Major Findings:

New data for calendar year 1970, covering over 2,000 person-years of experience as coded by this Section, have been added to the master tape and checked out for removal of detectable errors.

Many listings of data required by the Section have been prepared as requested. In response to a request by this Section as a responsibility of the contractor, a study of the relationship between fertility and usual menstrual cycle length, using a methematical model, is well advanced. An interim publication is expected.

The nature of seasonal cyclicity in menstrual rhythm, superimposed on changes associated with age, has been defined. A report for publication has been prepared.

Research on menarcheal age among native Alaskan girls attending BIA schools has been completed. Basic data for this project, gathered previously for this program by the Arctic Health Research Center under reimbursable agreement with the Institute, provide the first large-scale basis for this variable among natives in very cold climates. It was shown that menstrual cycles do not vary with the prolonged periods of light and darkness.

Complete programs needed for studies underway within this Section have been prepared as requested.

Significance of this contract to the Institute:

Maintenance of computer-processing operations at the University of Minn.

under contract continues to have several advantages of considerable assistance to the research program in menstruation and reproduction history, to wit: a) the small professional staff of 2 persons within the Institute has been freed of many routine tasks, enabling their concentration on design of investigations and writing the resulting reports for publication. Management of basic record flow, systematic filing facilitating immediate retrieval as is continually needed, coding the annual input of new histories, preparing visual aids, and corresponding as required with study participants, are maintained as functions of the Section necessitated by confidentiality of the basic records; b) the contractor had previously (1961-65) maintained all operations for this research program aside from those given above. This Institute has therefore acquired experience and skill in these operations at minimum cost to itself while maintaining effective collaboration in a basic research program for which the contractor had complete responsibility for over a quarter-century.

NICHD Annual Report
July 1, 1971 through June 30, 1972
Behavioral Biology Branch

Section on Brain and Behavior

The professionals in this Section include Drs. David Symmes, John Newman, Zvi Wollberg and Kenneth Chase.

1. Drs. John Newman, Zvi Wollberg and David Symmes have continued to utilize squirrel monkey vocalizations in analyzing auditory discriminative mechanisms in these animals. Analysis of single unit recordings in the auditory cortex of squirrel monkeys has shown that more than half of the neurons tested discriminate between pairs of acoustically similar vocalizations. Tape recordings of four natural vocalizations, representing different structural call groups, were each paired with another similar vocalization. The members of each pair of vocalizations differed in one or more aspects of acoustic structure but were within the range of acoustic variation for that call type: a) more than half of the 46 neurons tested responded to only one member of one or more pairs of calls; b) a significant number of cells responded with distinctly different discharge patterns to each member of a call pair. In another set of experiments, also recording from single neurons in this brain region, nine natural variations of a single call type (Isolation Peep) were used as acoustic stimuli. Seven of these variants were judged to be typical of this call type. The responses of 21 units tested with all nine vocalizations were analyzed: a) a majority (57%) of the neurons responded to all nine Isolation Peeps; b) another 19% responded to all seven "typical" Isolation Peeps, but not to one or the other atypical call. In only one case did a cell fail to give a clear response to one of the seven typical Isolation Peeps while responding to the rest; c) the remaining 24% of the units showed selective responsiveness to different subgroups of Isolation Peeps. Sound spectrographic analysis of sequences of vocalizations between a pair of squirrel monkeys suggest that the sounds made by one individual may influence the acoustic structure of the sounds made on that same sequence by the other individual.

Preliminary results from our behavioral training indicates that squirrel monkeys can discriminate between pairs of vocalizations representing the same call type. Duration and intensity do not appear to be major cues in these discriminations.

2. Drs. Symmes and Chase have completed behavioral and electrophysiologic observations on 5 control and 4 six-month isolation-reared rhesus monkeys. The experimental animals exhibit a severe and continuing emotional disturbance which manifests itself in individual and group directed behaviors, in the animals vocal repertoire and in their response to stress. Cognitive behavior such as problem solving appears less impaired or entirely intact. The EEG of the controls is normal with the possible exception of some deficiency of slow activity in sleep records. Chronic recordings of electrical activity from electrodes placed in limbic structures (amygdala, hippocampus

and septal region) indicate that these areas are functionally abnormal in the isolation reared animals. In contrast to the normal animals, ongoing spike activity in the hippocampus was seen in the isolates as well as an increased susceptibility to nystagmus evoked spike activity. Histologic confirmation of the recording sites is under way.

3. Analysis by Drs. Symmes and Chase of slow-wave electrical activity recorded from the surface of the monkey brain in conjunction with the performance of a timed motor response has continued. The major source of slow potentials recorded from the cortex of monkeys in a highly over-trained lever pulling situation is activity in the sensorimotor cortex contralateral to the hand used. A secondary source of electrical activity with a different distribution is associated with acquisition of a reward. The type of slow wave activity recorded in children making visual discrimination was not seen consistently in the monkeys and this animal model is probably not a favorable one for studying brain mechanism involved in the complex discrimination of meaningful stimuli.

Section on Neurobiology

The professionals in this Section include Drs. Phillip Nelson, Harold Gainer, Gerald Fischbach, John Peacock, Bruce Schrier, Ronald Fisk, Donald Shapiro, John Whysner and Herbert Levitan.

1. Dr. Harold Gainer has continued his studies of molluscan neurons in conjunction with Dr. Zvi Wollberg. In order to study both the synthesis of various cell constituents and control of axoplasmic transport of these constituents, considerable effort has gone into developing reliable intracellular injection techniques so that a variety of macromolecule precursors could be introduced into identified individual cells. The previously developed disc microelectrophoresis and a new ultra-micro isoelectric focusing system have been used on "soluble" and "insoluble" proteins separated from single cells by a microliter ultracentrifugation procedure. The low molecular weight proteins (5000 daltons) found in a bursting pacemaker cell in the land snail also occurs in an analogous cell with similar electrophysiologic properties found in the sea hare, Aplysia. Synthesis of other proteins which appear to be characteristic of specific groups of neurons in Aplysia has been demonstrated.

2. Drs. Nelson and Peacock have extended previous studies of continuous lines of neuronal and non-neuronal cells. Collaboration has been initiated with workers (Drs. Ruddle and McMorris) in the Department of Biology at Yale University. Hybrid lines of cells formed between neuroblastoma cells and either L-cells or a human diploid fibroblast line have been studied with regard to morphology, electrical excitability and chemosensitivity. Some neuroblastoma cells exhibit both excitatory and inhibitory responses to acetylcholine, and the receptors and ionic permeability mechanisms responsible for this differential chemosensitivity are distributed over the cell surface in a highly organized fashion. Acetylcholine applied to the cell body produces an excitatory effect while application to the cell processes produces an inhibitory effect. The neuroblastoma X human fibroblast hybrids have

considerable advantage over the previously studied hybrids from the standpoint of karyotypic and enzymatic markers of cell lineage. These hybrid lines exhibit a considerable range in their degree of functional differentiation suggesting that some variable loss of human or mouse chromosomes may have occurred. Recloning and karotyping of the hybrid lines is necessary to explore this possibility. Electrically, mechanically and chemically elicited active membrane responses of fibroblasts have been discovered and propagation of these responses between fibroblasts appears to occur. Large changes in cell membrane potential and permeability characterize this response and experiments are planned to explore the possibility that the electrical response may reflect some mechanism involved in regulation of cell mitosis or biosynthetic activity. Dr. Fisk has been developing methods for obtaining pure membrane preparations from the neuroblastoma, L-cells and hybrid lines. He has successfully adapted a method for selectively labelling cell membranes with ^{125}I in order to monitor the membrane separation procedures. Such properties as electrical and chemical excitability and the development of interneuronal communication are to a great extent a function of cell surface membranes. Cell membrane separation and analysis is therefore likely to be a crucial aspect of a number of neurobiologic studies and Dr. Fisk has made considerable progress in this regard. Some problems of membrane aggregation and contamination remain unsolved, however, and Dr. Fisk will be continuing his effort to solve these problems.

4. Drs. Peacock and Nelson have begun electrophysiologic studies on mammalian muscle, spinal cord and cerebellar cultures grown from material initially dissociated into single cells. Richly interconnected synaptic networks develop in these cultures and, as in the chick cultures described by Dr. Fischbach, differentiation of morphologic and electrophysiologic features of different neuronal types is maintained for many weeks in culture. Diverse forms of electrogenic membranes are manifestated in the chick and mouse muscle cells, spinal cord cells and dorsal root ganglion cells and these preparations should be favorable for the study of electrically excitable membranes (see below).

Attempts have been made to produce somatic cell hybridization between normal mouse neurons and human and mouse continuous cell lines. The preliminary results are encouraging, but the study of these putative hybrids has only just begun.

5. Dr. Gerald Fischbach working with Dr. John Whysner, Dr. Mark Dichter and Mr. Stephen Cohen has continued his experiments on avian muscle and nerve in tissue culture. They have shown and analyzed the distribution of acetylcholine (ACh) receptors along the cultured muscle fibers. Both electrophysiologic tests and radioautographic localizations of labelled α-bungarotoxin (a toxin which specifically binds to ACh receptors) have been used to locate the ACh receptors. The receptor distribution in both innervated and non-innervated fibers is patchy with peaks of the receptor density tending to be associated with the location of hypolemmal muscle nuclei. Relatively little change in the mean "background" ACh sensitivity is seen in muscle following innervation by spinal cord neurons.

Spinal cord and sensory ganglion neurons do not divide in culture since they show no evidence of DNA synthesis (tritiated thymidine incorporation). Action potentials in the spinal cord cells are blocked by tetrodotoxin (TTX) and the amplitude of the action potentials is dependent on the concentration of extracellular sodium ions. By contrast the dorsal root ganglion cells in culture generate action potentials which are not blocked by TTX and which are largely dependent on the extracellular calcium ion concentration. The neurites of these cells, however, develop action potentials whose mechanism is quite similar to that of the spinal cord neurons. Differential control, both of the synthesis of membrane constituents responsible for action potential generation and of the disposition of these membrane constituents over the surface of the cell, therefore occurs in these dorsal root ganglion cells. More than 50% of the neurons show electrical evidence of rich synaptic connections and EM studies done by Dr. J. Heuser on the spinal cord cultures show mature synaptic structures. Dorsal root ganglion cells grown in the presence of muscle cells are larger than such cells grown in the absence of muscle cells, suggesting a "trophic" interaction between these two cell types. Such metabolic interaction between the different cellular constituents of the culture (muscle, spinal cord neuron, dorsal root ganglion neuron and possible fibroblastic or glial elements) are of great interest and will be pursued by electrophysiologic, anatomic and enzymatic or biochemical techniques.

6. Drs. Bruce Schrier and Donald Shapiro, in collaboration with Dr. Alfred Gilman, have continued biochemical investigation of cultures of rat brain cells and clonal lines of rat glial tumor cells.

Surface cultures of fetal rat brain were found to be a reproducible system for the study of biochemical events in the developing nervous system. Two culture media were evaluated for their effect on these cultures; one was found to enhance neuronal differentiation while the other promoted cell growth. Growth of cultured cells, as measured by DNA and protein accumulation, was favored by relatively high culture densities. A neuron-specific enzyme, choline acetyltransferase (CAT) was synthesized preferentially in lower density cultures suggesting that multiplication of CAT-producing cells (presumably neurons) might be occurring. Studies with an inhibitor of DNA synthesis (5-fluoro-deoxyuridine) showed that neuronal multiplication was indeed occurring and that such multiplication was favored at low culture densities and in brain tissue from younger rat fetuses.

Fetal rat brain cell cultures were found to respond to treatment with β-adrenergic agonists, adenosine and prostaglandin E_1 by the accumulation of large concentrations of intracellular cyclic AMP. The β-adrenergic response was studied in detail and found to be similar in many respects to that of clonal rat glial tumor cells. Preliminary data suggest that the adenosine response is a neuronal contribution in brain cell cultures. The origin of the prostaglandin response has not been determined, although it has been shown that mouse neuroblastoma cells respond only to this agonist. Our data show that growth of brain cells in N^6-monobutyryl-cyclic AMP resulted in an induction of the enzymes acetylcholinesterase and glutamate decarboxylase. The significance of these phenomena and their possible relationship to the above cyclic AMP responses are under investigation.

Glutamic acid and γ-aminobutyric acid (GABA) metabolism were studied in clonal rat glial tumor cells. The data show that: a) glial cells have active mechanisms for catabolism of glutamate, a putative excitatory neurotransmitter; b) they contain glutamate decarboxylase (GAD) enzyme activity, which forms the inhibitory neurotransmitter GABA; c) "GAD II" activity of glial mitochondria, reported by Haber, Kuriyama and Roberts, was absent in brain cell cultures and all glial lines tested, and was shown to be a spurious result of these workers; d) glial cells actively concentrated two putative inhibitory neurotransmitters from the medium, GABA with low affinity and taurine with high affinity; e) glial cells excreted large proportions of synthesized and concentrated GABA back into the medium; the rate of this excretion was not affected by β-adrenergic agonists, indicating that the large increases in cyclic AMP which are produced by catecholamines were not coupled to GABA excretion. It was concluded that glial cells have an important contribution to the modulation of brain function.

7. Dr. Herbert Levitan, in conjunction with Dr. Jeffery Barker, has been studying basic mechanisms of ion specific membrane permeability. Their approach has been to make a correlation between the structures of a series of non-narcotic analgesics, such as salicylate, and their activities with regard to altering neuronal membrane permeability. They have found 1) the salicylates and related non-narcotic analgesics increase membrane permeability to potassium and decrease chloride permeability; 2) a good correlation exists between the effectiveness of these compounds in altering neuronal membrane permeability and their relative analgesic potency; 3) the effect of a compound on membrane permeability is related to the degree to which its structure would allow nonspecific binding or solubilization in the membrane. These compounds probably act by increasing the net negative charge on the neuronal membrane and this charge density is apparently a primary determinant of membrane permeability to ions.

Serial No. HD-BB1
1. Behavioral Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Neural correlates of multi-sensory reaction time behavior in monkeys.

Previous Serial Number: Same

Principal Investigator: David Symmes, Ph.D.

Other Investigators: Kenneth Chase, M.D.

Cooperating Units: None

Man Years:

Total:	0.3
Professional:	0.3
Others:	0.0

Project Description:

Objectives: To develop an understanding of the origin and significance of slow cerebral potentials associated with cognitive processing through the use of infrahuman primate models.

Methods Employed: Data collection in this project was substantially complete by the beginning of fiscal year 1972. Computer analysis of data on hand was the only effort expended on this project during the current year.

Major Findings: A pre-eminent role of the sensorimotor cortex in generating slow potentials during the actual movements associated with timed lever pulling and releasing has been confirmed. The existence of other sources of slow potentials unrelated to movement is not challenged by this finding, but the repeatability and exploitability of these "cognitive processing" indicators in such a monkey preparation appear to be at a low level. Our data suggest that several reports in the literature describing slow potential correlates of expectancy or memory retrieval in the monkey are speculative in that accurate description of motor behavior during the time period in question was not available.

Significance to Biomedical Research and the Program of the Institute: The behavior and cerebral recordings studied in this project are analogs of procedures widely used in adults and children to assess CNS function, and successful development of an infrahuman primate model would permit greater experimental manipulation of the relevant variables.

Proposed Course: This project will be discontinued during fiscal year 1972.

Honors and Awards: None

Publications: None

Serial No. HD-BB2
1. Behavioral Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies of CNS functioning in isolation reared monkeys.

Previous Serial Number: Same

Principal Investigator: David Symmes, Ph.D.

Other Investigators: Kenneth Chase, M.D.
John D. Newman, Ph.D.

Cooperating Units: Children's Hospital, Department of Neurology, Washington, D.C.

Man Years:

Total:	2.2
Professional:	1.6
Others:	0.6

Project Description:

Objectives: To describe alterations in CNS function, if any, which are associated with the isolation-reared syndrome in monkeys.

Methods Employed: The original group of 4 isolation reared and 4 mother-reared control rhesus monkeys was augmented by the addition of one control. These 9 animals were implanted with monopolar stainless steel glass insulated electrodes at age approximately 30 months. The electrodes were directed at targets within the limbic system (surface cortical electrodes were also placed in some animals) on the basis of extensive prior behavioral studies carried out in our laboratory and elsewhere. These data implicated brain systems involved in regulation of emotional behavior as sites of possible abnormal activity.

Recordings were made in these implanted preparations under several conditions and the resultant tape recorded electrical data subjected to a number of analyses, both hand and computer assisted. From 6 to 15 hours of records were taken from each animal. When the recording phase of the study was concluded, 8 of the animals were tested with non-injurious electrical stimulation of limbic structures to obtain information about the thresholds and spread of localized epileptiform activity (after discharges). The animals have been sacrificed in a painless manner and the location of the electrodes is being verified by standard histological techniques.

Major Findings: Electrical activity recorded from deep brain structures of the isolation reared group showed several abnormal features. Two of these were seen with higher consistency than others: 1) spontaneously occurring slow negative spikes in the hippocampus (the reference electrode was a large area steel screw in the skull) which were increased both in frequency of occurrence and in the degree of spread of the abnormality during excitement and/or stress. Spread was observed to occur between one hippocampus and the contralateral hippocampus but not to amygdala or septum. Both the restricted area of involvement and differential fatiguability of the abnormal activity contrast with the properties of somewhat similar hippocampal spikes induced by electrical stimulation of most limbic sites, and support the conclusion that a separate population of neurons is involved in the normal, electrically elicited epileptiform activity on the one hand and in the abnormal, spontaneous slow spiking we observed on the other. 2) The abnormally raised animals showed a greater tendency for electrical rhythms in several limbic structures to follow or be influenced by the periodic vestibular drive associated with caloric nystagmus. The differences between isolate and control groups in this "following response" is relative rather than absolute, and the role of eye movements is very complex and difficult to control. The evidence obtained in this pilot group of animals suggests that repetitive eye movements unrelated to vestibular drive (optokinetic nystagmus) do not influence brain rhythms substantially in either group. Caloric nystagmus does affect most limbic structures in the isolates, and this effect appears maximal in the septum.

Significance to Biomedical Research and the Program of the Institute: The severity and persistence of the behavioral abnormalities associated with isolation rearing are well documented, yet little or no data exists on the neurochemical or neurophysiological mechanisms which may be causally related. Some insight into these mechanisms could have wide applicability in the diagnosis and treatment of certain childhood disorders, particularly autism.

Proposed Course: To replicate and extend these findings in a new group of isolation reared monkeys; to investigate the age of onset of limbic abnormalities as seen in electrical recordings; to utilize neurochemical techniques for more precise description of the serotonin abnormality seen at an early age; and to relate the electrical and chemical findings.

Honors and Awards: None

Publications: None

Serial No. HD-BB3 (1)
1. Behavioral Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Morphologic and biochemical studies of nervous system cells in culture.

Previous Serial Number: Same

Principal Investigator: Bruce K. Schrier, M.D., Ph.D.

Other Investigators: Donald L. Shapiro, M.D.

Cooperating Units: Alfred G. Gilman, M.D., Ph.D., Department of Pharmacology, University of Virginia, Charlottesville, Virginia.
E. J. Thompson, M.D., Ph.D., Laboratory of Biochemical Genetics, NHLI

Man Years:

Total:	2.5
Professional:	2.0
Others:	0.5

Project Description:

Objectives: 1) To evaluate the biochemical, morphologic, and electrophysiologic events temporally surrounding the formation and function of synapses. 2) The importance of glial contributions to nervous system function. Culture of nervous system cells is to be used as a means to decrease the complexity of nervous system preparations. To this end a reproducible culture system of normal brain cells was developed and evaluated.

Methods Employed: Methods for growing brain cells in culture and assaying for nervous system marker enzymes have been described previously. Assays for intracellular cyclic AMP were performed by the method of Gilman.

Major Findings: 1) Surface culture of fetal rat brain cells was found to be a predictable system for study of biochemical events in the developing nervous system. Of two culture media evaluated, one was found to enhance neuronal differentiation, while the other promoted cell growth. Growth of cultured cells, as measured by protein and DNA accumulation, was a density-dependent phenomenon; however, disproportionately high levels of activity of the enzyme choline acetyltransferase (CAT) were found as inoculation densities decreased. This suggested that some neurons within the cultures (CAT-producing cells) might be capable of multiplication. Studies with an

inhibitor of DNA synthesis (5-fluoro-deoxyuridine) showed that significant neuronal division was indeed occurring. This phenomenon was enhanced by low inoculation densities and by use of younger fetuses as the source of the inoculum.

2) Fetal rat brain cell cultures were found to respond to treatment with β-adrenergic agonists, adenosine, and prostaglandin E_1 with the accumulation of large concentrations of intracellular cyclic AMP. The β-adrenergic response was studied in detail and found to be similar in many respects to that of clonal rat glial tumor cells. Preliminary data suggest that the adenosine response is a neuronal contribution in brain cell cultures. The origin of the prostaglandin response has not been determined, although it has been shown that mouse neuroblastoma cells respond only to this agonist. Our data show that growth of brain cells in N^6-monobutyryl-cyclic AMP resulted in an induction of the enzymes acetylcholinesterase and glutamate decarboxylase. The significance of these phenomena and their possible relationship to the above cyclic AMP responses are under investigation.

3) Glutamic acid and γ-aminobutyric acid (GABA) metabolism were studied in clonal rat glial tumor cells. The data show that: a) glial cells have active mechanisms for catabolism of glutamate, a putative excitatory neurotransmitter; b) they contain glutamate decarboxylase (GAD) enzyme activity, which forms the inhibitory neurotransmitter GABA; c) "GAD II" activity, reported by Haber, Kuriyama and Roberts to be present in glial mitochondria, was absent in brain and all glial lines tested, and was shown to be a spurious result of those workers; d) glial cells exhibited uptake of two putative inhibitory neurotransmitters from the medium: GABA with low affinity and taurine with high affinity; e) glial cells excreted large proportions of synthesized and concentrated GABA back into the medium; the rate of this excretion was not affected by β-adrenergic agonists, indicating that the large increases in cyclic AMP in response to treatment with catecholamines were not coupled to GABA excretion. It was concluded that glial cells may have an important contribution to the modulation of brain function by controlling the levels of neuro-active substances in the milieu of neurons.

Significance to Biomedical Research and the Program of the Institute: These studies represent the groundwork for investigations of the interaction of various glial and neuronal cells in the developing nervous system. Such interactions are major problems in neurobiology and an understanding of the genetic and biochemical control mechanisms involved in their regulation will be of relevance to the diagnosis and treatment of nervous system diseases.

Proposed Course: We feel that we now have sufficient expertise in growth and biochemical characterization of crude cell preparations from the nervous system to enable us to begin to purify individual cell types. Attempts at such purification will include the use of gradient centrifugations, differential attachment to surfaces, and enzymatic and agglutinin modifications of membrane properties. When purified cell lines are obtained, the genetic and biochemical control of their developmental interactions may be studied by a combination of techniques available in the Behavioral Biology Branch.

Honors and Awards: None

Publications:

1. Wilson, S. H., Schrier, B. K., Farber, J. L., Thompson, E. J., Rosenberg, R. N., Blume, A. J. and Nirenberg, M. W. Markers for gene expression in cultured cells from the nervous system. J. Biol. Chem., in press.

2. Schrier, B. K., Wilson, S. H. and Nirenberg, M. W.: Neuroblastoma and other cells from the nervous system. In Fleischer, S., Packer, L. and Estabrook (Eds.): Methods in Enzymology, in press.

3. Gilman, A. G. and Schrier, B. K.: Cyclic AMP in fetal rat brain cell cultures: I. Effect of catecholamines. Molec. Pharmacol., in press.

Serial No. HD-BB4
1. Behavioral Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Information processing in the central auditory system of mammals.

Previous Serial Number: Same

Principal Investigator: John D. Newman, Ph.D.

Other Investigators: Zvi Wollberg, Ph.D.
David Symmes, Ph.D.
Kenneth Chase, M.D.

Cooperating Units: Mrs. Garland Riggs (Professional Services Contract)

Man Years:

Total:	1.9
Professional:	1.7
Others:	0.2

Project Description:

Objectives: This project is concerned with the processing of complex sounds by the primate cerebral cortex and related brain structures. Special attention is being paid to sounds used by primates in intra-specific communication, to determine which acoustic cues are used by the brain and the animal to distinguish between the various elements of the communication repertoire.

Methods Employed: 1) Single unit recording: Action potentials are recorded from single cells in the auditory cortex of unanesthetized squirrel monkeys. Neuronal discharges are analyzed for excitatory or inhibitory effects of vocal stimuli. Response patterns are compared between groups of acoustically related vocalizations, and the various artificial models of communication sounds. Selected segments of vocalizations can be electronically removed to test the effects of different components on neuronal responses. Electronic sound generating instruments can be manually or automatically (under computer guidance) controlled, to generate artificial models of natural vocalizations.

2) Sound spectrographic analysis: A sound spectrograph is used to produce frequency versus time plots of the vocalizations used as acoustic stimuli greatly facilitating judgements as to the differences in acoustic features between these natural sounds. This technique is also being used to assess the transfer of information by vocalizations between pairs of monkeys.

3) Behavioral methods: Squirrel monkeys are being trained to respond in a shock-avoidance situation to one of a pair of acoustically similar vocalizations. These training techniques are being developed to assess this species' tendency to discriminate or generalize between groups of natural vocalizations. Equivalence testing is being used in an attempt to reveal the acoustic features this species uses to categorize its own communication repertoire into natural groups.

Major Findings: Analyses of single unit recordings in the auditory cortex of squirrel monkeys has shown that more than half of the neurons tested discriminate between pairs of acoustically similar vocalizations. Tape recordings of four natural vocalizations, representing different structural call groups were each paired with another similar vocalization. The members of each pair of vocalizations differed in one or more aspects of acoustic structure but were within the range of acoustic variation for that call type: a) more than half of the 46 neurons tested responded to only one member of one or more pairs of calls; b) a significant number of cells responded with distinctly different discharge patterns to each member of a call pair. In another set of experiments, also recording from single neurons in this brain region, nine natural variations of a single call type (Isolation Peep) were used as acoustic stimuli. Seven of these variants were judged to be typical of this call type. The responses of 21 units tested with all nine vocalizations were analyzed: a) a majority (57%) of the neurons responded to all nine Isolation Peeps; b) another 19% responded to all seven "typical" Isolation Peeps, but not to one or the other atypical call. In only one case did a cell fail to give a clear response to one of the seven typical Isolation Peeps, while responding to the rest; c) the remaining 24% of the units showed selective responsiveness to different subgroups of Isolation Peeps. This selectivity was not in most cases, clearly related to obvious acoustic differences between variants.

Sound spectrographic analysis of sequences of vocalizations between a pair of squirrel monkeys suggest that the sounds made by one individual may influence the acoustic structure of the sounds made in that same sequence by the other individual.

Preliminary results from our behavioral training indicates that squirrel monkeys can discriminate between pairs of vocalizations representing the same call type. Duration and intensity do not appear to be major cues in these discriminations.

Significance to Biomedical Research and the Program of the Institute: Our finding that neurons in the auditory cortex can discriminate between acoustically similar vocalizations opens up the possibility that these small differences in acoustic structure are behaviorally significant to individuals of this species. Preliminary findings in behavioral training studies likewise suggest that the acoustic differences found in variants of a single call type are discernible by the behaving animal. If further evidence substantiates our preliminary finding that this species uses subtle acoustic cues in intraspecific communication, this would indicate that a relatively sophisticated communication system may exist in this nonhuman primate. The ways in which

the nervous system and the behaving animal categorize these sounds is relevant to understanding the cerebral mechanisms in humans controlling the perception and categorization of speech sounds.

Proposed Course: Four fruitful lines of research are indicated: 1) analyzing vocal dialogues in pairs of monkeys to substantiate the use of subtle cues in social communication; 2) behaviorally conditioning monkeys using natural and artificially synthesized vocalizations as discriminanda to study the ways in which the behaving animal discriminates and categorizes between communication sounds; 3) testing single neurons with groups of acoustically similar vocalizations to determine the ways in which neurons categorize and discriminate between communication sounds; 4) studying the influence of other brain systems in controlling the response specificity of cortical neurons to vocalizations.

Honors and Awards: None

Publications:

Wollberg, Z. and Newman, J. D.: Auditory cortex of squirrel monkey: response patterns of single cells to species-specific vocalizations. Science 175: 212-214, 1972.

Serial No. HD-BB5
1. Behavioral Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Neurobiologic studies of neuronal and other cell types in cell culture.

Previous Serial Number: Same

Principal Investigators: Phillip G. Nelson, M.D., Ph.D.
John H. Peacock, M.D., Ph.D.

Other Investigators: John A. Whysner, M.D., Ph.D.
Harris R. Fisk, M.D.

Cooperating Units: Department of Biology, Yale University, New Haven, Connecticut.

Man Years:

Total:	6.8
Professional:	3.8
Others:	3.0

Project Description:

Objectives: To describe neurobiologically interesting properties of continuously dividing and normal cells in tissue culture, to elucidate the molecular basis for these properties, and to analyze the control mechanisms regulating their expression.

Methods Employed: Intracellular microelectrode techniques have been used to assay the neurophysiologic properties of the cell lines under investigation. Culture conditions have been worked out for maintaining mammalian nervous and muscle tissue in vitro. Cell hybridization techniques have been extended so as to utilize mouse-human hybrids in neurobiologic studies. Somatic cell hybridization techniques have been applied to normal neuronal and various continuous cell types. Radioactive labelling of cell membranes has been accomplished so that a marker for cell membranes is available for cell fractionation studies. Radioactive α-bungarotoxin, a small protein which selectively labels the acetylcholine receptor molecule, has been prepared and used in radioautography and binding studies.

Major Findings: Expression of neuronal properties in neuroblastoma x L-cell hybrids has been confirmed in series of hybrids derived independently from those studied earlier. Acetylcholine (ACh) sensitivity can be enhanced by manipulation of culture conditions and has been shown to occur in the hybrid

cell lines. Differential distribution of ACh receptors and their associated ionophores over the neuroblastoma or hybrid cell surface has been demonstrated. Mouse-human hybrids also exhibited some expression of neuronal properties; considerable heterogeneity of cell characteristics in the mouse-human clones was evident. Mammalian neuronal and muscle cells can be maintained in culture using material dissociated into single cells as the initial innoculum. The cells in these mammalian cultures develop cell specific morphologic and electrical properties and rich synaptic interconnections. Fibroblasts exhibit an active electrical response which can be elicited by chemical (ACh), mechanical and electrical stimuli and this response can be propagated from cell to cell. The ACh elicitation of the response but not the propagation is blocked by atropine.

Significance to Biomedical Research and the Program of the Institute: A wide range of neurobiologic properties are now available in a number of the tissue culture systems that we are studying. An understanding of the regulation and control of these properties is fundamental to the analysis of nervous system development and functioning. The application of the understanding of normal neurobiologic processes in experimental situations to problems of human nervous system development are of course problematical, but this area would seem to be of central concern to the Institute and the possible usefulness of the experimental work is considerable.

Proposed Course: The development of cell fractionation methods has begun and early results have been obtained with normal and continuous line cell hybridization techniques. Biochemical analyses of surface membranes and other cell constituents are planned. Separation of different cell types in the normal neuronal preparations will be attempted. The goal of these procedures is to understand this basis for the establishment of specific intercellular connections.

Honors and Awards: None

Publications:

1. Peacock, J., Minna, J., Nelson, P. and Nirenberg, M.: The use of aminopterin in selecting electrically active neuroblastoma cells. Exptl. Cell Res., in press.

2. Nelson, P. G., Peacock, J. and Minna, J.: An active electrical response in fibroblasts. J. Gen. Physiol., in press.

Serial No. HD-BB6
1. Behavioral Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Synapse formation between nerve and muscle cells in tissue culture.

Previous Serial Number: Same

Principal Investigator: Gerald D. Fischbach, M.D.

Other Investigators: Phillip Nelson, M.D., Ph.D.
Michael Baytipps
Stephen A. Cohen
John Whysner, M.D., Ph.D.

Cooperating Units: Maryanna Henkart, Ph.D., Laboratory of Neuropathology and Neuroanatomical Sciences, NINDS
Mark Dichter, M.D., Electroencephalography Branch, NINDS

Man Years:

Total:	2.8
Professional:	2.2
Others:	0.6

Project Description:

Objectives: Our long-term objective remains the study of neuron and muscle cell differentiation and synapse formation in cell culture. We plan to continue study of the sequence of events during nerve-muscle junction formation, of factors that regulate postsynaptic membrane chemoreceptors,, of the degree and mechanism of specificity in interneuronal synapse formation and of the development of different types of electrically excitable membranes. In each of these areas, ultrastructural and biochemical correlates of electrophysiologically defined phenomena will be sought.

Methods Employed: Conventional cell culture and intracellular microelectrode techniques remain the backbone of our experimental approach. Relatively purified cultures (muscle and nerve cells) have been obtained by the use of drugs that kill dividing cells and a "low growth" culture media.

Autoradiographic and electron microscopic techniques have been tailored to the cell culture system. Time-lapse cine studies of growing neurites and muscle cells are underway. Histologic techniques include silver, acetylcholinesterase and methylene blue stains.

Major Findings: 1) Nerve-muscle cultures. Innervation does not reduce the extrajunctional ACh sensitivity or lead to focal accumulation of the enzyme AChase near nerve terminals (two "neurotrophic" phenomena). Relative peaks of chemosensitivity were found in the region of nerve endings on innervated fibers but identical peaks were found over uninnervated muscle cells. In the latter case, the peaks were usually over or adjacent to hypolemmal muscle nuclei. The ACh response is blocked by low doses of α-bungarotoxin, and the uneven distribution of chemosensitivity is paralled by an uneven distribution of grains following autoradiography with I^{125} α-bungarotoxin.

2) Spinal cord and sensory ganglion cells. Spinal cord cells form rich synaptic networks in cell culture in which more than 50% of the cells receive spontaneous excitatory or inhibitory input. Synapses can be directly visualized after methylene blue staining or with Nomarski optics. They appear quite mature in electron micrographs. The neurons are not invested by glia. Neurons dissociated from 6-7 day embryos do not synthesize DNA in vitro and hence probably do not divide. Sensory ganglion (DRG) cells form synapses with spinal cord neurons and, perhaps, with each other. In the ganglion cells, a significant fraction of the inward current through the soma membrane is carried by Ca^{++} ions during the action potential, whereas the processes are more, if not exclusively dependent on Na^{+} ions. The large range in DRG cell sizes and neurite conduction velocities suggests that the cultures are composed of several types of sensory cells. DRG cells co-cultured with muscle fibers appear larger than controls which suggests a "trophic" interaction between the two cell types.

Significance to Biomedical Research and the Program of the Institute: The mechanism of synapse formation is a basic question in embryology. Formation and interuption of synaptic coupling between nerve and various nerve and muscle cells has profound structural and metabolic consequences. The tissue culture technique offers considerable hope for analyzing this striking biologic phenomenon. Modification of synaptic function by changes in activity pattern would help in understanding mechanism of neuronal plasticity or learning. Before asking the relevant biochemical questions, the basic electrophysiological phenomena must be understood. Tissue culture has provided a convenient system in this regard.

Proposed Course: We want to determine if chronic stimulation of muscle changes the distribution of ACh sensitivity over the muscle membrane and, if successful, to dissociate mechanical and electrical factors with drugs and hypertonic solutions. The "receptor" distribution will be followed by iontophoresis of ACh and with fluorescein conjugated α-bungarotoxin and, in collaboration with V. Marchesi (NIAMD) with freeze-etch techniques. Transsynaptic flow of labeled proteins and nucleotides will be examined by autoradiography. ACh receptors will be further characterized by determining the kinetics of α-bungarotoxin binding (with J. Whysner) and by attempting to distinguish junctional from extrajunctional receptors with physiologic techniques. The earliest stages of synapse fromation will be studied for evidence of electrical coupling and to determine the degree of correlation between ACh sensitivity and muscle nuclei and site of innervation. An explanation for peaks of sensitivity will be sought with electron microscopy

techniques and α-bungarotoxin labeled with an electron opaque marker.

Attempts will be made to isolate "pure" populations of identified neurons by density gradients, sepharose-receptor agonist complexes, differential adhesion on culture surfaces and by micro-dissection of embryos of various ages. The degree of specificity and plasticity of interneuronal synapses will be examined and agents that might affect synapse formation (such as enzymes and lectins) will be assayed. Studies of the membrane properties - including chemosensitivity - of individual neurons will be continued. Different types of sensory ganglion cells will be sought especially those that form spindles when cultured with muscle. The trophic interaction between sensory ganglion cells and muscle fibers will be further documented.

Honors and Awards: None

Publications:

1. Fischbach, G. D., Nameroff, M. and Nelson, P. G.: Electrical properties of chick skeletal muscle fibers developing in tissue culture. J. Cell Physiol. 78: 289-300, 1971.

2. Fischbach, G. D.: Synapse formation between dissociated nerve and muscle cells in low density cell cultures. Developmental Biol., in press.

Serial No. HD-BB8
1. Behavioral Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Correlative neurochemical and electrophysiological analyses of individual, identifiable neurons in Molluscan nervous systems.

Previous Serial Number: Same

Principal Investigator: Harold Gainer, Ph.D.

Other Investigators: Zvi Wollberg, Ph.D.

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.5
Others:	0.5

Project Description:

Objectives: To study the mechanisms by which the physiological activities of identifiable neurons influence the synthesis and axoplasmic transport of specific neuronal proteins.

Methods Employed: Several methods to separate and analyze proteins synthesized by single neurons have been developed in this laboratory. These include a microdisc polyacrylamide electrophoresis technique in sodium dodecylsulfate (Anal. Biochem., 1971), a conventional disc electrophoresis system on the microscale, and an ultramicro isoelectric focusing method (in preparation). These methods are able to detect as little as 10^{-10} grams of protein, and have been very successful in evaluating molecular weight and isoelectric point distributions of proteins from single neurons that were incubated in isotopically labeled amino acids. A technique for the ultracentrifugation of microliter quantities has been developed and used for the separation of "soluble" and "insoluble" fractions from single cells.

Two experimental preparations are being utilized for these studies. The isolated brain of the land snail, Otala lactea, and the abdominal ganglion of the sea hare, Aplysia californica. Both nervous systems contain large, identifiable neurons which are appropriate for combined electrophysiological-neurochemical studies. Conventional electrophysiological recording and stimulating methods are also employed in these studies.

A considerable effort has been directed towards developing a reliable intracellular injection technique for the purpose of injecting labeled amino acids into single identified neurons. In this way it is possible to label only one neuron in the ganglion with isotope, and hence any labeled proteins appearing in the peripheral nerves could be interpreted as being due to axoplasmic transport from the injected cell. Electrophoretic analyses of the proteins synthesized in the soma of a particular neuron, and evaluation of its axonal proteins would further characterize the selective biochemical properties of individual neurons. At present we are utilizing a pressure-injection system, in which a double-barreled glass microelectrode is used to penetrate a given neuron. One barrel contains 3 M KCl for recording membrane potentials, and the other barrel contains about 1-2 nanoliters of the material to be injected by air pressure (20-80 lbs/in^2). The tip of the double electrode is large, usually 2-4 microns, and therefore this method is applicable only to the larger neurons of Aplysia. The advantages of the pressure-injection method over iontophoresis are: 1) a large quantity of isotope may be injected rapidly, and 2) non-ionized materials and mixtures of substances with varied pH values may be injected.

Major Findings: Investigations of the cell-specific patterns of protein synthesis in five identified snail neurons, and two Aplysia neurons utilizing the microelectrophoresis in SDS method revealed that both cells R_{15} (in Aplysia) and #11 (in Otala) contained specific low molecular weight proteins (5000 daltons). These proteins were not present in the other cells tested, and could be correlated with the bursting spontaneous electrical activity of cells R_{15} and #11 and the neurosecretory nature of these cells. The 5000 dalton protein peak was absent in cell 11 during aestivation of the animal (see Anal. Biochem., 1971; Brain Res. 1972 a, b, c). Thus, individual neurons were shown to have unique patterns of protein synthesis, and one protein class appeared to exhibit sufficient plasticity to warrant a study of the effects of electrical and synaptic activity on its synthesis.

In an attempt to widen the search for neuron-selective protein synthesis, the patterns of synthesis for 6 cell types in Aplysia were evaluated by isoelectric focusing. Preliminary results suggest some selective patterns. Cells R_{3-13} appeared to have a unique basic protein (pI.8.5), while cells R_{15}, L_{2-6}, and the Bag cells appeared to synthesize a class of proteins at pI 5.0 not synthesized by the acetylcholine producing cells L_{11} and R_2.

Significance to Biomedical Research and the Program of the Institute: The approach described above is specifically designed to correlate relevant biochemical changes taking place in the nervous system with its specific functional activity. The heterogeneity of nervous tissue makes the most direct approach to this problem a desirable one. In this approach individual, identifiable neurons can be studied electrically and biochemically thereby providing a direct correlation between these events, and it is hoped ultimately an insight into what membrane phenomena in neurons lead to long-term intraneuronal biochemical changes. Finally, the biochemical micromethodology being developed will be of use in other areas of developmental biology where only small samples of material are available.

Proposed Course: The major thrust will be to apply specific neural input (either direct electrical and/or synaptic activation) to the cell types in which we have identified specific proteins. The aim will be to demonstrate modulation of specific protein synthesis by natural physiological influences, and to then determine the membrane mechanisms which are coupled into the intracellular biochemical control systems. With the intracellular injection method we aim to extend the study of the biochemical effects of neural activity to the problems of selective axoplasmic flow of proteins, and possibly transynaptic transport of proteins.

Honors and Awards: None

Publications:

1. Gainer, H.: Micro disc electrophoresis in sodium dodecylsulfate. Anal. Biochem. 44: 589-605, 1971.

2. Gainer, H.: Patterns of protein synthesis in individual identified molluscan neurons. Brain Research, in press.

3. Gainer, H.: Effects of experimentally induced diapause on the electrophysiology and protein synthesis patterns of identified neurons. Brain Research, in press.

4. Gainer, H.: Electrophysiological behavior of an endogenously active neurosecretory cell. Brain Research, in press.

Serial No. HD-BB9
1. Behavioral Biology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The effect of non-narcotic analgesic on the electrophysiological properties of neuronal membranes.

Previous Serial Number: New Project

Principal Investigator: Herbert Levitan, Ph.D.

Other Investigators: Jeffery L. Barker, M.D.

Cooperating Units: Electroencephalography Branch, NINDS

Man Years:

Total:	1.0
Professional:	1.0
Others:	0.0

Project Description:

Objectives: To study the physico-chemical basis for the effect of non-narcotic analgesics on neuronal membrane electrical and permeability properties, and thereby determine the mechanisms of action of non-narcotic analgesia.

Methods Employed: Standard electrophysiological techniques for intracellular recording and stimulation were employed to study the effect of these drugs on the electrical properties of large identified neurons in the buccal ganglion of the marine mollusc Navanax.

Major Findings: 1) that these agents decrease the output from a neuron and the effectiveness of input to it by increasing neuronal membrane permeability to potassium and decreasing chloride permeability; 2) a good correlation between the relative effectiveness of a drug in altering a neuron's electrophysiology and its relative analgesic potency; 3) that an acidic environment, known to be associated with pathologic and experimentally induced pain, causes a decrease in potassium permeability relative to chloride permeability, thereby exciting these neurons, and that this condition is effectively antagonized by salicylate; 4) in a structure-activity study the relative potency of compound is closely correlated with its relative ability to non-specifically adhere to or become soluble in the neuronal membrane; and 5) that the alterations which these organic molecules produce in the cation and anion permeability of the neuronal membrane are apparently due to

increasing the density or strength of the negative charge on the neuronal membrane; a consequence of adsorption of organic acids.

Significance to Biomedical Research and the Program of the Institute: We propose that the above results provide a physico-chemical explanation for the mechanism of action of non-narcotic analgesics such as aspirin. Moreover the high correlation found between the structure of salicylate analogues and their activity provided a basis with which one can predict the activity of as yet untested drugs and design drugs with any desired activity. On a more basic level it provides support for one of the current hypothesis concerning the control of ion permeability through biological membranes. The consequences of the latter are more far reaching than analgesia, and may provide a basis for understanding the effects of these drugs on a wide range of cell activity, such as metabolism and growth.

Proposed Course: To be continued elsewhere.

Honors and Awards: None

Publications:

Barker, J. L. and Levitan, H. pH and Salicylate: Antagonistic effects on permeability of a neuronal membrane. J. Gen. Physiol., 1971, 58: 710.

NICHD Annual Report
July 1, 1971 through June 30, 1972
Laboratory of Molecular Genetics

The Laboratory of Molecular Genetics conducts research directed toward understanding the molecular processes involved in the transmission of genetic information from generation to generation and in the regulated expression of this genetic information in cells during growth and differentiation. A variety of model systems are under investigation in the laboratory, ranging from bacterial and animal viruses to complex vertebrate regulatory responses involving the synthesis of specific antibodies and the development of specific organ systems. Taken together, the laboratory joins active workers in the areas of genetics, biochemistry, immunology, virology and cellular biology to bring their skills to bear on problems of mutual interest. During the past year, the Laboratory of Molecular Genetics has joined with the former Developmental Biology Branch, thus strengthening both units. At present, the Laboratory is comprised of five research groups and their activities are described in order of the complexity of the biologic system with which research is primarily concerned.

A. Considerable progress has been made in investigations directed toward understanding the integration and excision of the bacteriophage λ into and from its host's chromosome. Continuing from the discovery, made by this group last year, that λ would integrate into a variety of positions on the E. coli chromosome if its normal attachment site is removed, it has been found that the Xis protein, a portion of its normal integrative apparatus, is required for this abberant integration. Moreover, it has been shown that this abberant integration and excision occur with great precision, such that normal function is restored to a variety of transducing λ phage have been selected using these techniques and should prove of great value in studying regulated expression of genetic information encoded in the E. coli chromosome.

B. Closely related studies have been directed toward understanding the regulation of macromolecular synthesis, particularly the transcription process in E. coli. These studies have focused on the role of ppGpp, a unusual nucleotide discovered and identified by this group, and purified RNA polymerase and its effectors, sigma, Rho and psi factors. The nucleotide, ppGpp, accumulates in cells under conditions of amino acid starvation, a starvation which results in the coupled cessation of both ribosomal and transfer RNA biosynthesis. Mutant E. coli which fail to exert this coupled control, also fail to accumulate ppGpp. In studies using whole cells and cells made permeable to phosphorylated nucleotides, it has been found that ppGpp is in a state of dynamic equilibrium, that it is synthesized from a precursor which is not GTP, that its synthesis is stimulated by GTP, but that its synthesis also is dependent upon the presence of mRNA, and upon the synthesis or integrity of some elements of the cell's membrane.

A major effort has been directed toward elucidating a specific role for ppGpp in the regulation of transcription. The nucleotide does appear to inhibit RNA polymerase, particularly reducing the number of RNAs initiated by GTP. In addition, ppGpp has several pleiotropic effects on enzymes involved in nucleotide biosynthesis and transport, as well - and most interestingly - on enzymes

involved in protein biosynthesis and the replication of RNA viruses. It is tempting to compare this ubiquitous nucleotide - whose function remains to be elucidated - to cyclic-AMP, whose function so profoundly effects regulatory process in both bacteria and higher organisms.

C. Studies directed toward understanding the regulated expression of genetic information in higher organisms have taken advantage of an efficient, tRNA dependent cell-free system developed by this group last year. The system, derived from mouse ascites tumor cells, has been shown to translate accurately mRNA derived from the encephalomyocarditis virus as well as mRNAs corresponding to rabbit, human and avian globin and a mouse myeloma (immunoglobin) light chain. This assay permits the detection of very small amounts of biologically active mRNA.

This assay, in conjunction with a hybridization chromatography technique developed by this group, has been used to purify mRNA corresponding to rabbit, human and avian globin as well as that of myeloma light chain. Advantage was taken of the fact that the globin mRNAs contain a sequence of adenylic acid residues at their 3'-OH ends, to anneal a small oligo(dT) molecule to this template and to transcribe it into DNA using the avian myeloblastosis virus reverse transcriptase. Thus, DNA complementary to a mammalian gene was synthesized in vitro. This material should prove an extremely useful tool in experiments directed toward measuring gene dosage and specific mRNA synthesis as well as in efforts directed toward transmission of specific genetic traits from cell to cell, in vitro.

D. Studies at the molecular and cellular level were conducted in order to understand the process of differentiation in embryonic chick lens fibers. Explanted lens epithelium cells will undergo elongation (a step in normal lens development) if excised from 15 day old embryos. This developmental change has been found to be inversely related to the miotic and DNA synthetic activity of the cells. RNA synthesis, in contrast, is greatly increased in cells undergoing elongation and changes in the specific proteins synthesized by these cells are consistent with differentiation toward synthesis of lens crystallins. These studies also emphasize the importance of the microtubules in the cellular elongation process. These must be intact for rapid developmental changes to occur and must be synthesized in order that secondary developmental changes occur.

An additional, important, discovery of this group has been that insulin will substitute for serum in the culture medium of elongating cells. This permits the use of more carefully defined media and should permit studies requiring a more precisely controlled environment.

This group has alos investigated RNA synthesis in the mitochondria of unfertilized and fertilized sea urchin eggs. Fertilization is accompanied by an increase in both stable (largely ribosomal and tRNA) and unstable RNA synthesis. Reminescent of the observations noted above with respect to ppGpp, an unusual nucleotide has been discovered in unfertilized eggs. The ability to synthesize this nucleotide resides in the mitochondria of that egg, but requires a particle-free supernatant from unfertilized eggs for its synthesis.

E. Proceeding to even more complex biologic phenomena, studies have been directed toward understanding factors which effect the induction and cellular population dynamics in the immune response. These demonstrate that single immunoglobulin producing cells synthesize antibodies directed toward only one of several haptens presented simultaneously to the immunized animal. Other studies implicate a role for cyclic-AMP in amplification of the immune response which is presumed to occur through the action of T-cells. Finally, and potentially of great importance, specific classes of sheep anti-rabbit IgG immunoglobulins have been identified which possess "IgG-enhancing" activity and specific serum fractions have been isolated which promote antibody formation in rabbit spleen cell cultures.

In conclusion, the Laboratory of Molecular Genetics has undergone considerable growth and change in the past year, additional strong appointments to its staff will occur in the coming year and the forthcoming location of all the Laboratory's facilities in a single site, Building 6, should contribute to its productivity in the years to come.

Serial No. HD-LB16
1. Laboratory of Molecular Genetics
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Control Mechanisms in Temperate Bacteriophage
I. The control of normal phage integration and excision
II. Secondary pathways of phage integration and excision
III. The physiology of abnormally excised phage

Previous Serial Number: Same

Principal Investigator: R. Weisberg

Other Investigators: K. Shimada
N. Sternberg

Cooperating Units: Laboratory of Molecular Biology, Section on Biochemical Genetics, NCI

Man Years

Total:	24/12
Professional:	24/12
Others:	0

Project Description:

Objectives: To understand the mechanism of the insertion of virus DNA into and its excision from the chromosome of its host; to understand the control the phage exerts over the synthesis of proteins which are required for these processes; to study the nature of abnormal phage insertion and excision with the aims of understanding the normal process, on the one hand, and of determining the feasibility of using abnormal insertion as a tool for biochemical analysis of bacterial gene products, on the other.

Methods Employed: The organisms of study are bacteriophage λ, other genetically related phages, and their host, the bacterium E. coli. Genetic techniques are being used to obtain cells which carry virus DNA inserted at abnormal chromosomal sites; from such abnormal lysogens, we obtain novel types of transducing phage lines (phage strains whose chromosomes contain DNA of bacterial origin). Phage mutants with specific chromosomal aberrations are being used to characterize the activity and stability of the factors which govern insertion and excision. Temperature sensitive control mutants are being used to study the regulation of the synthesis of these factors.

Major Findings: (1) Normally, the DNA of phage λ is inserted into the bacterial chromosome at a specific chromosomal site called an attachment site. We have found that insertion still occurs, although at reduced frequency, in mutant bacteria which have lost the normal attachment site (see last year's report). Our continuing studies of abnormal insertion have allowed us to further define the action of xis protein, a phage specific product which is required for normal prophage excision. Briefly, we have found that xis protein is also required for a number of other types of recombination between attachment sites, and we believe that xis protein is always required for attachment site recombination when certain nucleotide sequences are present in a specific configuration. (2) In collaboration with the LMB/NCI, we have used a transducing phage line isolated from an abnormal lysogen to partially purify a bacterial protein which controls the activity of a group of genes that function in the metabolism of the sugar galactose (Parks, et. al). (3) Occasionally, abnormal lysogens are found in which insertion has occurred within a bacterial gene, thereby inactivating its function. In these cases excision of the phage DNA generally restores normal gene function. We have mapped the precise location of the phage DNA in two such lysogens and have found that in both cases the insertion point is in a region which encodes the amino acid sequence of a bacterial protein. Thus, the fact that excision restores normal gene function suggests that it restores the original sequence of nucleotides as well. (4) We are developing a method which has considerable potential for the generation of specific kinds of transducing phage lines. Briefly, the method entails isolating the phage particles liberated by a mixed bacterial culture consisting of many different abnormal lysogens. Such a phage stock contains many different types of transducing phages. By this method we have already isolated a phage that carries bacterial DNA believed to encode the amino acid sequence certain ribosomal proteins.

Significance to Biomedical Research and the Program of the Institute: The insertion and excision of viral (and other episomal) DNA is known to change the physiology of the host organism. In addition, these viruses (and other episomal elements) provide an important mechanism of genetic transfer and exchange in the microbial world. It is highly likely that such a successful symbiotic relationship has an analogy in the relationship between certain animal viruses and the cells of higher organisms. Moreover, an understanding of the control mechanisms operative in these easily studied microorganisms has obvious relevance to an understanding of regulatory elements in higher organisms. The ability to direct the insertion of phage DNA to predetermined locations on the host chromosome should prove to be an extremely useful and widely applicable method for isolating bacterial gene products and studying the control of bacterial gene expression.

Proposed Course of Project: We intend to further characterize the mechanism of phage DNA insertion into abnormal sites and its relation to normal insertion. We hope to construct new transducing phages for regions of the bacterial chromosome that are of particular interest. We plan to continue studies initiated several years ago on the mechanism of inactivation of the xis protein and the properties of a phage λ variant which is unable to cyclize its DNA. Dr. Sternberg plans to continue his studies of a bacterial mutant that is unable to synthesize stable RNA at high temperature and that is unable to propagate phage λ.

Honors and Awards: None

Publications:

1. Weisberg, R. and Gottesman, M.: The stability of Int and Xis functions. In Hershey, A.D. (Ed.): The Bacteriophage λ. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory of Quantitative Biology, 1971, pp. 489-500.

2. Gottesman, M. and Weisberg, R.: Prophage insertion and excision. In Hershey, A.D. (Ed.): The Bacteriophage λ. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory of Quantitative Biology, 1971, pp. 113-138.

3. Kellenberger-Gujer, G. and Weisberg, R.: Recombination in bacteriophage λ. I. Material exchange of DNA during Int-, Red- or Rec-promoted recombination. In Hershey, A.D. (Ed.): The Bacteriophage λ. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory of Quantitative Biology, 1971, pp. 407-416.

4. Shimada, K., Weisberg, R., and Gottesman, M.: Prophage Lambda at unusual chromosomal locations. I. Location of the secondary attachment sites and the properties of the lysogen. J. Mol. Biol. 63: 483-503, 1972.

5. Parks, J , Gottesman, M., Shimada, K., Weisberg, R., Perlman, R., and Pastan, I.: Isolation of the gal repressor. Proc. Nat. Acad. Sci., U. S. A., 68: 1891-1895, 1971.

Serial No. HD-LMG-1
1. Laboratory of Molecular Genetics
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Integrative Control of Macromolecular Synthesis

Previous Serial Number: NDS(I)-68 LMB/RC 1552

Principal Investigator: Michael Cashel, M.D., Ph.D.

Other Investigators: H. A. Raué, Ph.D.
Robert Crouch, Ph.D.

Cooperating Units: Department of Genetics, University of Washington
Department of Biological Sciences, Columbia University
Biological Laboratories, Harvard University
Laboratory of Biochemistry, NHLI, NIH

Man Years

Total:	2.9
Professional:	1.9
Others:	1.0

Project Description

Objectives: The broad objective of this project is to understand the mechanisms by which cells are able to coordinately adjust their composition in response to changes in physiological conditions and thus avoid cell injury and death. In this regard particular attention is again given to the role played by an unusual guanine nucleotide, abbreviated ppGpp. The discovery of this compound, its chemical characterization and biological regulation have been described in earlier project reports. This project is currently subdivided into three interest areas: 1) biosynthesis of ppGpp, 2) regulation of DNA dependent RNA transcription by ppGpp and 3) the effects of ppGpp as a regulatory nucleotide with inhibitory metabolic effects.

Methods Employed: In addition to methods described earlier, three modifications of existing methods mahve been devised which might be of more general utility. 1. Protein purification. Protein precipitates (such as obtained with protamine or ammonium sulfate) are immobilized in a celite matrix and controlled dissolution is achieved with gradients. In this manner virtually homogeneous preparations of RNA polymerase can be obtained in a day whereas usually a week is required. Final purification is achieved with ordinary ion-exchange or sizing column chromatography. 2. Permeabilized cells. Cell preparations have been obtained by abrupt temperature and ionic strength change. Such cells retain the capacity for synthesis of RNA, protein, phospholipids and ppGpp when

appropriately supplemented yet have virtually complete removal of small molecular components and permeability barriers. 3. Phospholipid measurement. A method was devised which allows direct application of cells to thin-layer chromatograms and nevertheless allows classical phospholipid resolution techniques. This method eliminates the need for tedious phase extractions and increases the number of samples easily processed daily at least ten-fold.

Major Findings: I. Biosynthesis of ppGpp. 1. Synthesis of ppGpp in whole cells. Studies of phosphate labeling kinetics indicate that a phosphorylated precursor for ppGpp exists. This precursor is metabolically labile during exponential growth turnover characteristic approximately that of mRNA. No phosphates in ppGpp are derived from metabolically stable phosphorylated species, excluding donation from rRNA, tRNA, DNA and phospholipids. During derepressed ppGpp synthesis, a steady state of rapid synthesis and breakdown holds, maintaining ppGpp formation rates of about a half million molecules per second per cell. This rate is several orders of magnitude larger than the specific rate of initiation of RNA and protein chains. 2. ppGpp synthesis is dependent upon the presence of mRNA. The addition of rifampicin to growing or starved cells was shown by Wong and Nazaar to inhibit ppGpp accumulation during the stringent reponse, suggesting that RNA polymerase initiation might directly participate in ppGpp biosynthesis. We have extended these experiments and ruled out this interpretation by demonstrating that 1) actinomycin has an effect similar to rifampicin, 2) neither drug inhibits ppGpp synthesis under conditions of restricted tRNA aminoacylation and 3) rifampicin at 300 times the minimal inhibitory concentration for B. subtilis (1 μg/ml) does not affect ppGpp synthesis can be made largely dependent upon T7 phage RNA polymerase transcription of T7 DNA in the presence of rifampicin. This demonstrates that ppGpp synthesis requires a mRNA like transcript and that this requirement can be met by heterologous mRNA. The most simple interpretation of available evidence is that ppGpp biosynthetic reactions constitute an idling reaction in protein synthesis. This reaction is thrown into play when a deficiency of aminoacylated tRNA occurs and is dependent upon mRNA presumably by virtue of its attachment to ribosomes. Hence ppGpp biosynthesis is only indirectly dependent upon RNA polymerase activity. 3. ppGpp synthesis in permeabilized cells. Using permeable cell preparations described, ppGpp synthesis can be measured whereas it has so far not been seen in crude extracts. The synthesis of ppGpp is not due to contamination with whole cells since i) no uptake of nucleotides occurs, ii) the pools of ATP and GTP are reduced by more than 99%, iii) the specific activities of ppGpp synthesis are 2-10X higher than whole cells, and iv) synthesis is sensitive to antibiotics at low concentrations which are ineffective in whole cells. The synthesis of ppGpp is regulated much the same as in whole cells since it is dependent upon i) a deficiency of a single amino acid, ii) a deficiency of a charged tRNA species even when a full complement of free amino acids is present and iii) the stringent allele of the RNA control gene.

The addition of GTP stimulates the initial rate as well as the extent of ppGpp labeling with ^{32}P and at the same time evokes a stimulation of beta labeled GTP and GDP. Replacing GTP with GDP yields labeled GDP but not labeled GTP or ppGpp. Tritiated GTP as well as ^{32}P gamma, alpha, or beta GTP do not label ppGpp in the presence of cold GDP but tritiated GDP will label ppGpp in the presence of cold GTP. Replacing beta-gamma methylenyl GTP for GTP results in abolition of stimulation of ppGpp. Apparently the stimulatory effect of GTP

requires gamma hydrolysis but the GTP contributes neither the guanine ring nor phosphates to ppGpp. Stoichiometry of tritium labeling of ppGpp with tritiated GDP suggests the existence of a precursor for ppGpp in these preparations as in whole cells. When cells are uniformly labeled with ^{33}P, then shocked and incubated with an excess of cold Pi and GTP, isotope labeling of ppGpp occurs. The effect of GTP is to increase the rate of conversion of precursor to ppGpp rather than stimulate the rate of formation of precursor in shocked cells. Relaxed cells can synthesize precursor, judging by the small amount of ppGpp synthesized in these cell strains. Precursor synthesis is slightly stimulated by chloramphenicol (CAP) rather than inhibited. Therefore CAP and the rel interfere with ppGpp synthesis at the step of conversion of precursor to ppGpp. Inhibitors of membrane function, such as triton, toluene, freezing, phospholipase, etc., interfere with precursor synthesis. When the capacity to synthesize phospholipids is abolished, ppGpp synthesis is much reduced and vice versa. It is concluded that physiological synthesis of precursor is somehow related to membrane function. 4. Correlation between ppGpp synthesis, protein synthesis and phospholipids. Protein synthesis occurs in shocked cells at 10-30% the specific activities of whole cells and requires supplements. The most marked stimulation is obtained with GTP and amino acids. Optimization of beta-galactosidase activities reveal properties similar to cell lysates rather than whole cells. Optimization of supplements for protein synthesis leads to optimization for ppGpp synthesis with two exceptions which also apply to whole cell regulation: 1) an incomplete array of amino acids exceptioanlly inhibits protein synthesis while stimulating ppGpp synthesis and 2) CAP and other protein synthesis inhibitors can inhibit protein synthesis without stimulating ppGpp synthesis in minimally supplemented shocked cells is CAP resistant, while CAP sensitive in whole cells. When shocked cells are also supplemented with glucose, GTP and DPN (or TPN) then ppGpp synthesis is rendered CAP sensitive. Phospholipid synthesis in shocked cells is also dependent upon aminoacids and upon GTP supplementation (but not ATP). Generally these experiments indicate that ppGpp biosynthesis requires as a necessary but not sufficient condition that both protein ahd phospholipid synthetic capacities are intact. It is inferred that these activities are also integrated in whole cells.

II. Regulation of DNA dependent RNA transcription by ppGpp. 5. RNA polymerase inhibition in the absence of factors. Cells accumulating ppGpp clearly have altered transcriptional activity including inhibition of rRNA and tRNA synthesis, stimulation of some mRNA species and inhibition of other species. The question is whether any of these modifications are in fact physiologically mediated by ppGpp. Since ppGpp reaches levels that exceed three of the four substrates of RNA polymerase it seems quite possible that ppGpp does function in this role; yet compelling evidence is still lacking. The simple addition of ppGpp to RNA polymerase reactions does give a limited inhibition (25-50%) of overall activity. This inhibition is largely due to blocking initiation of a portion of RNA chains beginning with GTP while ATP starts are not severely affected. Binding studies indicate ppGpp binds mole for mole with RNA polymerase core with binding characteristics identical to those reported by Wu and Goldthwaite for the initiation site. Since ppGpp neither binds to elongation sites nor is incorporated into the 3' end of growing RNA chains, it seems the ability of ppGpp to specifically inhibit initiation resides in the specificity of the enzyme initiation site. By phosphate transfer experiments measuring the

labeling of ppGpp with alpha ^{32}P UTP and CTP after alkali it is apparent that ppGpp inhibits chains starting pppGpU...more severely than those starting ppGpC... This result is curious since the 3' pyrophosphate residue on ppGpp should block diester formation with UTP and CTP equally well if ppGpp is simply binding to the initiation site on the enzyme. In any event, ppGpp inhibition of transcription shows a sequence specificity; it is not yet clear whether the transcriptional modification in whole cells show a similar specificity. The effects of ppGpp on RNA polymerase in vitro and in vivo are reversible. 6. RNA polymerase inhibition in the presence of factors. The earlier suggestion that ribosomal transcription in vitro requires psi factor in addition to RNA polymerase (Travers, Kamen and Schlief, Nature '71) has been challenged recently (Haseltine, Nature '72). However, the Haseltine result has in turn also been challenged (Murooka and Lazzarini, Nature '72) who found little rRNA transcript in the absence of added factor. Accordingly the earlier observations that ppGpp preferentially inhibits psi stimulation of rRNA synthesis are in question. It will be necessary to unequivocally demonstrate the enzymatic basis of rRNA transcription in vitro before it can be reasonably asked whether ppGpp participates in inhibition of this transcriptional event. Despite this, there are a number of interesting recent developments concerning the identity and properties of psi factor. Psi factor derived from subunits III and IV of phage Qβ replicase have been proposed by the Harvard group as identical to Tu and Ts transfer factors in protein synthesis. In collaboration with Dr. Blumenthal we have examined the binding of a series of guanosine di-, tri- and tetraphosphate isomers substituted at the 5' and 2' or 3' positions; these compounds were first synthesized in this laboratory. These studies indicate that ppGpp binding to psi is specific for the 5' pyrophosphate residue and that further 2' or 3' substituents had no effect. The binding of GDP and ppGpp are mutually competitive and about 100X tighter than for GTP which also binds to this site. It is noteworthy that the 3' end of Qβ RNA is very similar to the 3' end of tRNA and that Qβ replicase activity with poly C templates is severely inhibited by both GDP and ppGpp. 7. ppGpp effects on coupled systems. Transcriptional effects of ppGpp are also evident in crude systems in which DNA transcription is coupled to mRNA translation. Pastan's laboratory (NCI) has observed that ppGpp stimulates the transcription of beta galactosidase in crude coupled systems but inhibits the transcription with partially purified RNA polymerase. Zubay has demonstrated that this stimulation by ppGpp is abolished in mutants carrying a deletion such that the lac operon is under control of the tryp promotor. This clearly demonstrates promotor specificity and therefore transcriptional modification by ppGpp. Zubay has also demonstrated that tryptophan activities and galactokinase synthesis are inhibited by ppGpp in coupled systems whereas arabinose synthesis is greatly stimulated. The synthesis of tRNA in coupled systems, using a biological assay, is apparently unaffected by low concentration of ppGpp (0.1 mM); the effects of concentrations present during the stringent response are currently being studied in collaboration with Dr. Zubay.

III. Inhibitory effects of ppGpp on metabolism. 8. The extent of cellular metabolic inhibition during the stringent response. There is evidence that cells have a rather pleiotropic capacity to limit their biosynthesis activities when confronted with a depletion of a carbon-energy source or a required amino acid; both conditions are associated with ppGpp accumulation. The list of processes inhibited include synthesis of rRNA, tRNA, ATP, GTP, phospholipids,

phosphate esters (glycolytic) and after a time, DNA. The ability of cells to transport nucleosides, nucleobases and alpha-methylglucosides across cell membranes is also impaired. In each instance, mutants lacking the ability to accumulate ppGpp also fail to show impairment of the biosynthetic of transport process. The existence of such mutants is not unique to E. coli for they also occur in Salmonella and in Bacillus subtilis strains. We have accordingly begun to ask which of these inhibition, if any, might be attributed to a pleiotropic inhibitor function of ppGpp. 9. The effects of ppGpp on purine biosynthesis. Cells accumulating ppGpp invariably show a halving of the GTP pools together with a severe restriction in the conversion of IMP to GMP. Similar but less severe effects occur at the level of ATP synthesis. In collaboration with Dr. Gallant and Dr. Irr, it has been possible to show that ppGpp directly inhibits IMP dehydrogenase activity but not XMP aminase, as well as to inhibit adenylosuccinate synthetase. The inhibition constants obtained for the two enzymes can account for the inhibitory activities shown by cells. Further, since neither IMP nor its precursors accumulate in cells undergoing the stringent response, an earlier inhibition site in de novo purine synthesis seems very likely. 10. The effects of ppGpp on purine nucleoside and base transport. Recently purine phosphoribosyltransferases have been implicated in the membranous transport of purines in E. coli; separate enzymes catalyse the transport of adenine and guanine forming 5' monophosphates inside the membrane. Since cells with high levels of ppGpp show a 20-fold impairment of GTP labeling with labeled guanine and a 3-4 fold impairment of ATP labeling with labeled adenine, the effects of ppGpp were examined in this system in collaboration with Dr. Ozer (NHLI). We found that ppGpp inhibits: 1) membrane transport of 6-OH purines with a Ki of about 0.1 mM, 2) membrane transport of adenine with a Ki of about 2 mM and 3) purified phosphoribosyltransferase preparations which were not membrane bound. Relaxed mutants which do not show transport defects had transport systems as inhibitable by ppGpp as normal cells. Membranous transport of nucleosides was as inhibitable as purines supporting the contention that nucleosides are cleaved to the free base prior to transport. We infer that ppGpp could mediate purine transport defects shown by cells during the stringent response. 11. Anomalous stability of nucleotidyl phosphate during the stringent response. Whole cell phosphate labeling studies reveal that a cold phosphate chase imposed at the time of amino acid starvation does not chase uniform label from ATP, UTP, CTP or ppGpp whereas it does chase gamma label from GTP. This phenomenon has been demonstrated in the stringent member of several isogenic stringent-relaxed pairs of strains; in each case the relaxed strain shows that all nucleotides can be chased under similar conditions. The very rapid turnover of GTP, but not ATP, is anomalous since both protein synthesis and RNA synthesis inhibition is expected to slow the turnover rates of ATP and GTP equally. Our working hypothesis is that cells derive energy from a substrate phosphorylation with GDP as the primary phosphate acceptor rather than oxidative phosphorylation of ADP.

Significance to Biomedical Research and the Program of the Institute: Integrative control processes are a characteristic of normal cells; aberrations of these controls in the general sense might constitute a definitition of differentiation and development. Extension of microbial regulation to eukaryotic cells might be plausible in some form, as particularly evident from the work of Dr. G. Tompkins, who has pointed out the virtually invariable correlations between the effects of the stringent response in bacteria with the effects of

serum starvation in cultured human cells. As yet, ppGpp or a compound with a similar function has yet to be found in higher cell systems, although the existence of such a regulator has been proposed by Tomkins.

Proposed Course of Project: We hope to learn the enzymatic basis of ppGpp biosynthesis as well as its effects upon gene transcription and intermediary metabolism. Further attempts will be made to establish the presence of ppGpp in a eukaryotic system. Chemical synthesis of related analogs of ppGpp continues in hopes of obtaining further evidence of the structural basis of ppGpp activity and of possibly obtaining stable pharmacologic agents with actions similar to ppGpp.

Honors and Awards: None

Publications:

1. Gallant, J., Irr, J. and Cashel, M.: The mechanism of amino acid control of guanylate and adenylate biosynthesis. J. Biol. Chem. 246: 5812-5816, 1971.

2. Hochstadt-Ozer, J. and Cashel, M.: The regulation of purine utilization in bacteria V: Inhibition of purine phosphoribosyltransferase activities and purine uptake in isolated membrane vesicles by guanosine tetraphosphate. J. Biol. Chem., in press.

Serial No. HD-LMG-2
1. Laboratory of Molecular Genetics
2.
3. Bethesda

NIH-PHS
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Factor Influencing Genetic Transcription - Initiation and Termination

Previous Serial Number: None

Principal Investigator: Robert J. Crouch, Ph.D.

Other Investigators: None

Cooperating Units: Cold Spring Harbor Laboratory, Cold Spring Harbor, New York - Dr. Walter Keller

Man Years

Total:	1
Professional:	1
Others:	0

Project Description:

Objectives: The study of the coordinate expression of several genes during the development of the bacteriophage T4 and lambda has led to many of the current concepts of gene regulation. Unfortunately many of these concepts have not gone beyond the theory stage. For example, a factor (rho), which can produce RNA molecules of unique and "appropriate" size in an in vitro system, could provide the key to "turning on" the class II RNA species. The objective of a part of this study is to determine the mechanism of activity of rho and how its activity can change after bacteriophage infection. A second objective is to develop new methods for examining transcription.

Methods Employed: The purification of rho activity is based on the procedure of Roberts and on an unpublished procedure of Mr. Ned Minkley (The Biological Laboratories, Harvard). RNA polymerase was a gift of Dr. M. Cashel. Ribonuclease H was purified by Dr. Walter Keller (Cold Spring Harbor Laboratory). RNA synthesis in the presence of ribonuclease was as described by Krakow (the measurement of formation of charcoal unadsorable ^{32}P from γ ^{32}P ATP). γ ^{32}P ATP was prepared as described by Penefsky and Racker. The products of RNAse H digestion were separated by thin layer electrophoresis on PEI sheets.

Major Findings: 1. Ribonuclease H isolated by Walter Keller of Cold Spring Harbor from KB cells has been shown to be an endonuclease producing acid soluble products of varying sizes, each of which is probably terminated in a 5' phosphate. The function of this ribonuclease is unknown but could prove to be a

useful tool in studying RNA molecules which are capable of binding to DNA to form stable DNA-RNA hybrids. 2. Purification of rho by the procedure developed by Mr. Ned Minkley has yielded, in my hands, an activity which is different than the rho activity reported by Roberts and by Minkley in that it fails to bind to phosphocellulose. By several other criteria this activity is identical to that of rho. Unfortunately, the phosphocellulose step is an important purification step which means the purity of the activity I am seeing is not great enough to compare the proteins directly with the enzymatic activity. It seems likely that part of the difference I see can be attributed to the pH during the DEAE column step or purification or to the DEAE cellulose itself. One important aspect of this finding should result in an increased yield of rho per unit of bacterial cells. 3. Rho fails to work in presence of ribonuclease. This suggests that the RNA of the DNA-RNA-RNA polymerase complex in RNA synthesis is necessary for rho activity. Another trivial explanation which is yet to be excluded is that rho is removed from the reaction mixtures used by the ribonuclease added.

Significance to Biomedical Research and the Program of the Institute: The regulation of transcription can be essential in controlling gene expression in most organisms. Certainly changes in gene activity mediated through regulation of transcription can affect normal cellular activity while failure to regulate properly or to reorient transcription can be the basis of many medical abnormalities.

Proposed Course of Project: To further purity "rho-like" activity and find the relationship to the rho activity of Roberts. Secondly, to establish the necessity of RNA from rho to be active and to see if there are proteins - working in conjunction with rho to "recycle" the RNA polymerase molecules. Also the changes in rho activity upon T4 infection will be studied.

Honors and Awards: None

Publications: None

Serial No. HD-LBC6
1. Laboratory of Molecular Genetics
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Protein Synthesis: Mechanism and Role in Regulation of Cell Growth
I. Role in bacterial growth and bacteriophage development
II. Role in the expression of specific mammalian genes

Previous Serial Number: Same

Principal Investigator: P. Leder

Other Investigators: H. Aviv
I. Boime
R. Callahan
S. Krauss
S. Packman
D. Swan

Cooperating Units: Viral Leukemia and Lymphoma Branch, NCI

Man Years

Total:	80/12
Professional:	68/12
Others:	12/12

Project Description:

Objectives: To define in genetic and molecular terms the mechanisms which regulate the flow of genetic information during cellular growth and development with special emphasis on the role of translation reactions in this process. To understand these processes in simple model systems which are ultimately relevant to an understanding of complex genetic regulatory functions in man.

Methods Employed: Several model systems have been selected for these studies. One involves the bacterium E. coli and one of its viruses, T7. Others involve the constitutively synthesized mammalian protein, globin, and an induced class of mammalian proteins, the immunoglobulins. Two cell-free systems, one from Escherichia coli, the other from the Krebs II ascites tumor, have been derived in order to translate and, thereby, detect mRNA during its isolation. Elements required for both these systems have been purified using conventional biochemical techniques. These systems, in turn, have been used to detect activity associated with mRNAs corresponding to the EMC virus, human, rabbit and avian globin, as well as to two mouse myeloma proteins. The mRNAs are purified using

standard protocols involving exploitation of their physical properties as well as by hybridization chromatography, a technique recently developed in our laboratory. The execution of these procedures requires the maintenance of animals, tumor cells grown in animals and in culture, as well as the growth and isolation of mammalian viruses. Isolated mRNAs are, in turn, converted into their radioactively labeled DNA complements for use in molecular hybridization experiments designed to measure gene dosage and mRNA synthesis.

Major Findings: 1) A cell-free system was derived from mouse Krebs II ascites tumor cells which was dependent upon the addition of tRNA. 2) This system was shown to translate accurately mRNA derived from the EMC virus and to synthesize discrete EMC proteins which would account for as much as 40% of the information on the EMC genome. 3) The EMC mRNA was found to be initiated *in vitro* from a single translational initiation point consistent with its behavior *in vivo*. 4) The cell-free system also directed the synthesis of authentic human, rabbit and duck globin in response to very small amounts of mRNA. 5) This property was used as a very sensitive assay to detect globin mRNA during its purification. 6) A new technique, hybridization chromatography on oligo-(dT)-cellulose, was devised to take advantage of the fact that mammalian mRNAs contain stretches of poly(A) and to purify these and other mammalian mRNAs. 7) The purified globin mRNAs were converted into their DNA complements using the avian myeloblastosis virus DNA polymerase and the resulting products were shown to have properties expected by the relevant mammalian genes.

Significance to Biomedical Research and the Program of the Institute: We are undertaking to understand how genetic information is expressed in a sequential manner, either over the long-range developmental scale or, over shorter time periods during induction of specific protein synthesis. The results of this work should yield information of central importance in the understanding of these processes in man. In addition, our current studies have provided a means of synthesizing at least a major portion of a mammalian gene *in vitro*. This product should prove very useful as a tool to test various model mechanisms designed to explain genetic regulation in health and disease. It may also prove useful in the direct transmission of genetic characteristics from cell to cell *in vitro*.

Proposed Course of Project: The radioactive DNA complement of globin mRNA will be used, a) to determine the level of globin gene dosage in cells committed to development as erythroblasts, b) to determine the level of specific globin mRNA in erythroblasts at various stages of development, c) to follow the course of globin mRNA synthesis from nucleus to cytoplasm, d) to attempt to measure the level of mRNA present in polysomes of patients with thallssemia, and e) to use this product in an effort to transform non-globin producing cells in culture. In addition, the cell-free system will be used in the purification of the myeloma protein mRNA which will be similarly employed to elucidate the mechanism of antibody biosynthesis in appropriately induced cells. Work directed toward understanding the biosynthesis of the protein synthetic elements in bacteria and in the translational control of expression of the late genes of the bacteriophage T7 will be continued. In this respect, new methods of polyacrylamide gel analysis and immunoassays will be applied to detect initiation factor synthesis in *E. coli* and in cells infected with appropriate transducing phage, hopefully carrying genes corresponding to these elements. In addition, the

male cells which fail to translate mRNA corresponding to the late genes of the bacteriophage T7 will be examined to determine the site of this translational discrimination.

Honors and Awards: None

Publications:

1. Aviv, H., Boime, I. and Leder, P.: Protein synthesis directed by encephalomyocarditis virus RNA: Properties of a tRNA dependent system. Proc. Nat. Acad. Sci., U.S.A. 68: 2303-2307, 1971.

2. Boime, I., Aviv, H. and Leder, P.: Protein synthesis directed by EMC RNA. II. The in vitro synthesis of high molecular weight proteins and elements of the viral capsid. Biochem. Biophys. Res. Commun. 45: 788-795, 1971.

3. Ross, J., Aviv, H., Scolnick, E. and Leder, P.: In vitro synthesis of DNA complementary to purified rabbit globin mRNA. Proc. Nat. Acad. Sci., U.S.A. 69: 264-268, 1972.

4. Leder, P.: The elongation reactions in protein synthesis. In Anfinsen, C.B. (Ed.): Aspects of Protein Synthesis, Part B. New York, Academic Press, in press.

5. Caskey, T., Leder, P., Moldave, K. and Schessinger, D.: Translation, its mechanism and control. Science, in press.

6. Aviv, H. and Leder, P.: Purification of biologically active globin mRNA by chromatography on oligo(dT)-cellulose. Proc. Nat. Acad. Sci., U.S.A., in press.

Serial No. HD-LMG3

1. Laboratory of Molecular Genetics
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Molecular Aspects of the Differentiation of Embryonic Chick Lens Fibers in vivo and in Tissue Culture

Previous Serial Number: HD-DB10
HD-DB10 (1)
HD-DB8
HD-DB2
HD-DB18
(HD-DB17 discontinued)

Principal Investigator: Joram P. Piatigorsky

Other Investigators: Sydney P. Craig, III
Sonia S. Rothschild
Miriam Wollberg
Henry deF. Webster

Cooperating Units: None

Man Years

Total:	2.8
Professional:	1.2
Others:	1.6

Project Description:

Objectives: Cells in the excised lens epithelium of the 6-day old chick embryo elongate in tissue culture if serum is present in the medium. In the absence of serum, the epithelial cells survive for at least 10 days but fail to elongate. Cell elongation in the cultured embryonic lens epithelium is a potentially useful model for the study of cellular differentiation, since the lens epithelial cells also elongate when they form fibers in vivo. The purposes of the present investigations were, (1) to establish that the cell elongation which occurs in culture resembles the differentiation of lens fibers with respect to DNA synthesis, mitotic activity, protein synthesis and ultrastructure, thus providing evidence that the culture system is a faithful model of normal embryonic development, (2) to explore the mechanisms of the changes which take place during lens cell elongation in culture with respect to gene activation, differential messenger RNA stability and utilization of microtubules and (3) to simplify the culture system by eliminating the serum requirement for cell elongation.

Methods Employed. Embryonic lens epithelia of white Leghorn chickens were excised and cultured at 37°C. For histology, explants were fixed with Carnoy's fixative, embedded in paraffin, sectioned serially in a plane normal to the surface of the epithelium and stained with hematoxylin and eosin. Mitotic indices and measurements of cell length were determined from the histological sections. For electron microscopy, tissues were fixed with glutaraldehyde, post-fixed with osmium tetroxide and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate and examined electron microscopically. Quantitative determinations of DNA synthesis were made after labeling epithelia with H^3-adenosine or H^3-thymidine and isolating the DNA by Sephadex chromatography, centrifugation in cesium chloride or precipitation with acid. The percentage of cells synthesizing DNA was determined autoradiographically from H^3-thymidine labeled epithelia. RNA was phenol extracted and examined on composite polyacrylamide-agarose gels after labeling epithelia with H^3-adenosine, H^3-uridine or H^3-methionine. Labeled nucleotides were examined by thin layer chromatography. The absolute amount of ATP was determined by the luciferin-luciferase reaction. The synthesis of specific proteins, labeled with H^3-valine, was studied by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isofocusing in polyacrylamide gels and in sucrose density gradients.

Major Findings: (1) Dependence on Embryonic Age for Lens Cell Elongation in Culture. Lens epithelial cells from embryos 15 days of age or less elongate in tissue culture, but those from 19-day old embryos do not. The fact that the epithelial cells from 19-day old embryonic lenses do not elongate in culture is consistent with the fact that these cells no longer give rise to lens fibers in vivo. (2) DNA Synthesis and Mitosis. DNA synthesis, the percentage of nuclei labeled with H^3-thymidine and the percentage of cells with mitotic figures decrease about 3-fold after 3 days of cultivation of 6-day old embryonic lens epithelia. By contrast, lens epithelial cells taken from 19-day old embryos show an increase after cultivation in DNA synthesis, in the percentage of nuclei labeled with H^3-thymidine and in the percentage of cells with mitotic figures. Thus, there is an inverse relationship between lens cell elongation in culture and cell division. DNA synthesis and mitosis are also both inhibited during the formation of lens fibers in vivo. (3) RNA Synthesis. Serum stimulates an approximate 2-fold increase in the incorporation of H^3-adenosine into polydisperse and 4 S RNAs and a 4-fold increase into 28 S and 18 S ribosomal RNAs. These values were normalized on the basis of the specific activity of the intracellular pool of ATP. Experiments with dactinomycin showed that the greater incorporation is not due to slower degradation of RNA. There is also a 4-fold increase in the rate of methylation of ribosomal RNA during the initiation of cell elongation in culture. The rate of methylation of 45 S precursor ribosomal RNA and the accumulation of methylated 32 S, 28 S and 18 S RNAs are all stimulated, but there is no affect on the rate or extent of labeling of the S-adenosylmethionine pool with H^3-methionine. Thus, the initiation of lens cell elongation in culture is associated with an increase in the synthesis of all types of RNA, especially of ribosomal RNA. (4) Protein Synthesis. The lens epithelium of 6-day old embryos synthesize proteins which range in molecular weights from more than 200,000 to less than 20,000. Major species of protein labeled in 4 hours have molecular weights of about 91,000, 65,000, 27,000 and 20,000 with a principal component of approximately 45,000 containing

some 35% of the new protein. After one day in culture, the epithelial cells double in length and synthesize a smaller proportion of protein with molecular weights of 91,000 and 65,000. Approximately 50% of the newly synthesized protein has a molecular weight of 45,000. This pattern of synthesis remains for at least 28 days as the cells elongate in culture. In the fibers of the intact 6-day old embryonic lens, 80% of the new protein has a molecular weight of 45,000. Thus, cells in the cultured lens epithelium undergo changes in protein synthesis that are characteristic of epithelial cells differentiating into lens fibers.

The protein with a molecular weight of 45,000 represents subunits of delta crystallin, the protein with a molecular weight of 27,000 represents subunits of beta crystallin, and the protein with a molecular weight of 20,000 represents subunits of alpha crystallin. Intensive synthesis of delta crystallin, then, characterizes lens fiber differentiation in vivo and in culture. Inhibition of RNA synthesis with dactinomycin severely inhibits the synthesis of proteins with molecular weights of 65,000 and of 91,000 and partially inhibits the synthesis of alpha and beta crystallins, but does not affect the synthesis of delta crystallin. These data suggest that unstable and stable messenger RNAs exist in the lens epithelium, assuming that the dactinomycin-induced decrease in synthesis of specific proteins is due to the degradation of their messenger RNAs. The unstable messenger RNAs have a half-life of 1 to 3 hours while delta crystallin messenger RNA has a half-life longer than 9 hours. (5) Ultrastructure and Microtubules. The differentiation of lens fibers in vivo and in tissue culture is associated with a decrease in organelle density, a clustering of ribosomes and an alignment of longitudinally oriented microtubules along surface membranes. Disruption of the microtubules with colchicine or vinblastine sulfate inhibits lens cell elongation in culture. Neither of these agents affects protein synthesis. In contrast to the effects of colchicine and vinblastine sulfate, cycloheximide inhibits protein synthesis but does not prevent the initial doubling of cell length, although it does suppress further elongation. Cycloheximide does not prevent the assembly and alignment of microtubules which take place during lens cell elongation. These results indicate that the early stages of cell elongation in the cultured lens epithelium require an initial assembly and organization of preexisting microtubular elements and that continued elongation depends, in addition, on the de novo synthesis of protein, possibly microtubule protein. (6) Stimulation of Cell Elongation by Insulin Rather than Serum. Insulin can substitute for serum to promote lens cell elongation in tissue culture. As during cultivation with serum, the nuclei become ovoid and tend to align in the center of the elongated cells during cultivation with insulin. In general, the magnitude of increase in cell length is directly proportional to the logarithm of the insulin concentration over the range of 10^{-3} µg/ml to 1 µg/ml. Ten µg/ml of insulin does not produce more cell elongation; rather, it appears to be less effective than 1 µg/ml.

Insulin suppresses DNA synthesis and mitosis in cultured 6-day old lens epithelia about 4 times more effectively than does serum. However, insulin and serum stimulate DNA synthesis and mitosis to the same extent in 19-day old embryonic lens epithelia.

Significance to Biomedical Research and the Program of the Institute: One of the difficulties of studying cellular differentiation is that embryonic systems have many unidentified variables and generally involve interaction of many cell types. The differentiation of lens fibers has the advantages that (1) it occurs synchronously in a single population of excised epithelial cells, (2) all processes examined in culture mimic those which take place *in vivo* during the normal development of lens fibers, (3) large amounts of easily identified lens specific proteins are involved and (4) simple morphogenetic changes can be related to biochemical events. The finding that insulin can stimulate lens cell elongation in a fully defined medium should facilitate further analysis of the mechanism of cell elongation. These studies, then, give information on the mechanisms of cellular differentiation, which is relevant to the goals of this Institute.

Proposed Course of Project: All aspects of this work will be continued. Emphasis will be placed on further characterization of lens crystallins, isolation of specific messenger RNAs, regulation of microtubule assembly, synthesis of microtubule protein and mechanisms of insulin action on cell elongation.

Honors and Awards: None

Publications:

1. Piatigorsky, J. and Rothschild, S. S.: Effects of serum on the synthesis of RNA and of DNA in the cultured lens epithelium of the chick embryo: Initiation of lens fiber formation *in vitro*. Biochim. Biophys. Acta 230: 86-98, 1971.

2. Piatigorsky, J., Webster, H. deF. and Craig, S. P.: Protein synthesis and ultrastructure during the formation of embryonic chick lens fibers *in vivo* and *in vitro*. Develop. Biol. 27: 176-189, 1972.

3. Piatigorsky, J. and Rothschild, S. S.: Loss during development of the ability of chick embryonic lens cells to elongate in culture: Inverse relationship between cell division and elongation. Develop. Biol., in press.

Serial No. HD-LMG4
1. Laboratory of Molecular Genetics
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Microchondrial RNA Synthesis in Unfertilized Eggs and Embryo of the Sea Urchin

Previous Serial Number: HD-DB9

Principal Investigator: Sydney P. Craig, III, Ph.D.

Other Investigators: Michael Cashel, M.D., Ph.D.
Joram Piatigorsky, Ph.D.
Lisa Prescott, M.D.*

Cooperating Units: *Section on Neurocytology, NINDS

Man Years

Total:	1.0
Professional:	1.0
Others:	0

Project Description:

Objectives: The mitochondria of sea urchin eggs have been shown to contain DNA which is transcribed during early development. The purpose of this project is to determine the types of RNAs present in, and synthesized by, mitochondria during oogenesis and after fertilization, and also to explore the synthesis of nucleotides in eggs, embryos, and isolated mitochondria in an effort to discover possible mechanisms for the regulation of mitochondrial nucleic acid synthesis.

Methods Employed: Nucleotides and nucleic acids were labeled during oogenesis by injecting ^{32}p and ^{3}H-uridine, respectively, into shed sea urchins and allowing the growing oocytes to incorporate the label. Labeled unfertilized eggs were obtained in intervals ranging from 3 days to 3 months after the injection of radioactivity. Embryos were labeled by incubation with ^{3}H-uridine, ^{3}H-adenosine, or ^{14}C-amino acids. Incorporation of radioactivity into RNA and protein was measured after TCA precipitation. In other experiments, labeled RNAs were extracted with phenol and analyzed by polyacrylamide gel electrophoresis, benzoylated DEAE-cellulose chromatography, and DNA-RNA hybridization. The soluble nucleotide pools in eggs, embryos and isolated mitochondria, labeled with ^{32}p, were extracted with formic acid or with a solution of sodium formate and were analyzed by thin layer, ion exchange chromatography. Mitochondria, isolated by sucrose buoyant density centrifugation from various stages of

embryonic development, were embedded and sectioned for observation in the electron microscope.

Major Findings: (a) Mitochondrial RNA synthesis during development: The mitochondria of unfertilized eggs contain a stable population of RNAs with molecular weights of approximately 700,000 and 350,000 (possibly mitochondrial ribosomal RNA) and of about 25,000 (possibly mitochondrial transfer RNAs). After fertilization or artificial activation, mitochondria synthesize a relatively unstable population of polydisperse RNAs and a relatively stable population of discrete RNAs. The relatively stable RNAs have molecular weights of 700,000, 350,000 and 25,000. RNA polymerase activity was not detected in intact, isolated mitochondria. Mitochondrial DNA synthesis was not apparent during early embryonic development (pregastrulation). (b) Labeling of nucleotides in eggs, embryos and in mitochondria isolated from various stages of embryonic development: An as yet unidentified nucleotide (spot 6) which migrates on ion exchange chromatography plates more rapidly than any of the nucleotide triphosphates, is synthesized in relatively large proportion in unfertilized eggs. The mitochondria, isolated from both fertilized and unfertilized eggs, appear to have similar capacities for labeling the various nucleotides. However, mitochondria from both fertilized and unfertilized eggs are stimulated by the 100,000 x g supernatant fraction, from unfertilized egg homogenates to label spot 6. ATP plus a methyl ester of leucine also stimulate the labeling of spot 6. The identification of spot 6 and its relation to the regulation of mitochondrial nucleic acid synthesis are presently under investigation. Magic Spot (ppGpp) does not appear to be a normal product of mitochondrial nucleotide synthesis. (c) Mitochondrial Ultrastructure: The mitochondria from unfertilized sea urchin eggs appear, in the electron microscope, to contain a regular array of cristae that is continuous with the inner mitochondrial membrane. Thickenings of the cristae, running through adjacent cristae, perpendicular to their plane, are also observed. Electron dense spheres of uniform diameter are occasionally found in the inner mitochondrial membrane.

Significance to Biomedical Research and Program of the Institute: The discovery of DNA in mitochondria has suggested that mitochondria may have a larger regulatory function than previously realized. Sea urchin eggs are especially suitable for the study of mitochondria, since (1) they have a large ratio of cytoplasm to nucleus, (2) they have a relatively low proportion of endoplasmic reticulum, and (3) nuclear ribosomal RNA synthesis is not initiated until gastrulation of the embryo. Also, as much of the biosynthetic apparatus of the mitochondrion is similar to that of bacteria, with respect to drug sensitivity and biochemistry, it appears reasonable to approach the study of the regulation of mitochondrial nucleic acid synthesis in a manner similar to which this subject has been approached in bacteria, where it has been shown that unusual nucleotides may have an important regulatory function in transcription. These studies are important for understanding mitochondrial genetic activity during the normal course of embryonic development.

Proposed Course of Project: This project will be continued with emphasis placed on the relationship of the labeling of nucleotides in embryos and in isolated mitochondria to the synthesis of nucleic acids and to the ultrastructure

of mitochondria in various stages of embryonic development.

Honors and Awards: None

Publications:

1. Craig, S. P.: Synthesis of RNA in non-nucleate fragments of sea urchin eggs. J. Mol. Biol. 47: 615-618, 1970.

2. Craig, S. P. and Piatigorsky, J.: Protein synthesis and development in the absence of cytoplasmic RNA synthesis in non-nucleate egg fragments and embryos of sea urchins. Effect of ethidium bromide. Develop. Biol. 24: 214-232, 1971.

3. Craig, S. P.: DNA, RNA and protein synthesis in anucleate fragments of sea urchin eggs. In the symposium volume of the International Symposium on Biology and Radiobiology of Anucleate Systems. Bonotto, S. (Ed.), Academic Press, 1972.

4. Piatigorsky, J.: "Gametogenesis" in The Sea Urchin Embryo: Biochemistry and Morphogenesis. Czihak, G. (Ed.), Springer-Verlag, in press, 1972.

Serial No. HD-LB20
1. Laboratory of Molecular Genetics
2.
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Regulation of Antibody Biosynthesis
I. Mechanisms of consignment of cells to determinant-specific immunocompetency
II. Promoters and amplifiers of antibody biosynthesis in cell culture
III. Initiation of determinant specific antibody biosynthesis in cell culture
IV. Deviation of immunocompetent cells

Previous Serial Number: Same

Principal Investigator: E. E. Hanna

Other Investigators: C. Lambert
A. Uzunova

Cooperating Units: None

Man Years

Total:	27/12
Professional:	27/12
Others:	0

Project Description:

Objectives: To discover and characterize pathways of antibody biosynthesis and secretion by antigen stimulated cells. Immediate attention is directed as follows: Toward understanding the role(s) of certain accessory non-secreting cells during initiation of an antibody response. Toward learning the nature and roles of promoter substances found in serous fluids which seem to be required to initiate a complete antibody response in cell culture. Toward determining whether promoter substances are normally derived from certain of the closely associated accessory cells such as macrophages and thymus derived cells (T-cells). As solutions to these problems develop, attention will be directed further toward devising means of selective regulation of cellular synthesis and/or secretion of preferred antibodies.

Methods Employed: Rabbit spleen cells are cultured in the presence of antigen by a procedure adapted from the Mishell-Dutton technique (Science 153: 1004, 1966). After 4 or more days in culture a large number of the cells have been stimulated to produce antibodies specific for the antigen. Antibody secreting cells are detected as plaque-forming cells (PFC), when they are

incubated subsequent to harvest from culture, in a lawn of indicator cells bearing determinants of the antigen. A determinant specific replica assay (J. Immunology 107; 172, 1971) allows the study of population dynamics and individual specificities of cells from culture or from intact immunized animals. An electronic biological freezer greatly facilitates these studies since cells may be frozen at various stages of an antibody response, and later recovered from liquid nitrogen with the activity which prevailed at the time of freezing. Antisera prepared against accessory cells, when used in the presence of complement can be used to eliminate certain lines of cells and other antisera prepared and characterized in this laboratory (manuscript in preparation) facilitate recognition of other lines of cells. Antigen specific columns are helpful for isolation and purification of specific populations of antibody secreting cells. Density gradients are also useful in this respect.

Some experiments are performed in intact animals rendered deficient of accessory or secretor cells by drugs, or cytotoxic antisera, or surgery. Animals genetically lacking of certain cells, e.g., thymus-less, nude mice, are also available. We are continuing to search for better cloning methods.

Major Findings: Experiments have just been completed demonstrating an absence of "bridge" or linkage antibody forming cells in populations previously shown to secrete antibodies specific for added determinants of synthetic immunogens (manuscript in preparation). This finding compliments our previous work in showing essentially absolute monospecificity of cells once they are induced to secretion, and suggests that the combining sites of the antibodies made have no effective overlap into regions of the carrier structure, i.e., none that can be detected biologically.

Other experiments have progressed using neonatally thymectomized mice which strongly suggest a role of amplification, through cyclic nucleotide mediators by T-cells (manuscript in preparation).

We have localized the "IgG-enhancing" activity (IgG-plaque development) of sheep anti-rabbit IgG antisera to particular classes of sheep immunoglobulins. The particular class appears to determine the overall facilitating or suppressing character of the antiserum. This study has involved column fractionation of several sheep antisera taken at various stages of immunization (manuscript in preparation). Concurrently, we have preliminarily localized the active components of sera which promote antibody formation in rabbit spleen cell cultures by column fractionation of active and inactive serum samples.

Significance to Biomedical Research and the Program of the Institute: Ability to regulate or modulate specific antibody responses and immune responses in general will have great advantage over current measures, since current measures employ cytotoxic drugs which induce generalized immunosuppression including desirable antibody responses to disease producing immunogens and suppression of immune surveillance in total. Findings of these studies should complement scientific thought on cellular control mechanisms in general.

Proposed Course of Project: We are continuing efforts to define functions of cells known to play some role in the complex event of specific immunoglobulin secretion. The macrophage function remains of central interest as one of the helper accessory cells. Whether it is an informational role or nutritional or yet another remains to be ascertained. Results outlined above relative to other accessory cells along with our study of the macrophage function, and the various serum promoter factors will be further delineated in cell culture. It is a concern of high priority to determine which functions are manipulable with respect to regulation.

Honors and Awards: None

Publications:

1. Hanna, E. E. and Merchant, B.: Secretion of monospecific anti-hapten IgG antibodies by rabbit immunocytes. J. Immun. 107: 172-184, 1971.

NICHD Annual Report
July 1, 1971 through June 30, 1972
Laboratory of Biomedical Sciences

The Laboratory of Biomedical Sciences conducts basic research in developmental enzymology, intermediary metabolism, physiological controls, developmental pharmacology, and molecular structure. The objective of these investigations is the elucidation of the molecular basis for selected types of mental and physical developmental pathology.

In the Section on Developmental Enzymology interest centers on the separation, purification, and characterization of enzymes from the human placenta and from fetal tissues in both normal and abnormal pregnancies. A study of the properties of pregnancy-associated enzymes from normal and abnormal gestations may give information about the etiology of reproductive disorders such as sponteneous or habitual abortions, stillbirths, or molar pregnancies.

Placental β-glucuronidase has been separated into two major isoenzymes and partially purified by a combination of ammonium sulfate fractionation, a heat step, and a combination of DEAE and CM chromatography. The administration of steroid hormones to nonpregnant females does not increase serum hexosaminidase isoenzyme B. This is evidence that the elevation of this serum enzyme in pregnancy is not mediated by steroid-producing organs such as the placenta or the ovary. The liver may be implicated in the elevation, since levels are increased in patients with icteric hepatitis. Recent work indicates that choriocarcinoma cells in culture take up β-glucuronidase and hexosaminidase isoenzymes from the serum supplements of the culture medium and that the cells simultaneously elaborate cell-specific hexosaminidase isoenzymes into the medium. Electrophoretic evidence was obtained that acid phosphatase in choriocarcinoma differs fundamentally from the enzyme in term placenta.

Investigators in the Section on Intermediary Metabolism develop laboratory analogs of mental retardation through the use of specific chemical agents and enzyme inhibitors, investigate the biosynthesis and degradation of macromolecules in neural tissue, elucidate mechanisms of transport of small molecules into and out of the brain, and evaluate the effects of excesses or deficiencies of intermediary metabolites on cellular differentiation and organogenesis (particularly of the brain). The studies in the Section range from classical biochemical investigations of enzymes of neurochemical interest and pharmacological efforts to inhibit or stimulate these enzymes to behavioral experiments on animals in which the activity of such enzymes has been altered or inhibited. In this way it is hoped to provide information on the basic biochemistry of the brain as well as on the conditions which alter the normal brain functions such as various genetically-transmitted mental retardation syndromes.

Among recent advances made by the Section are: (1) the demonstration that a model of clinical phenylketonuria can be produced in the rat by the administration of p-chlorophenylalanine plus phenylalanine (This model has been shown to be valid at the biochemical, behavioral, and pathological levels.), (2) the observation that various hydroxylase enzymes differ from one another in their cofactor specificities. (3) the finding that p-chlorophenylalanine

is activated to a small extent by E. coli and rat liver t-RNA-activating enzymes, (4) the demonstration that Comamonas sp. possesses two enzymes, both catalyzing the first step in the biosynthesis of the pteridine ring, (5) the development of a new isotopic assay for purine-phosphoribosyl transferases, (6) the study of the GMP-phosphoribosyl transferase from rat brain.

The Section on Physiological Controls is concerned with hormonal, nutrient, and neural regulation of mammalian cell and organ metabolism and with the development of corresponding regulatory systems. A central focus on synthesis and mode of action of cyclic AMP reflects the potential importance of this ubiquitous intracellular regulator to our understanding of metabolic regulation, cell growth, and differentiation. Specific studies are conducted on: (1) differential modulation of hepatic glucose output and adaptive protein synthesis by glucose, polypeptide hormones, and biogenic amines, and (2) neuronal control of biochemical pathways mediating indole and biogenic amine metabolism in a neuroendocrine tissue, in the pineal gland, and in the brain.

Recent studies have shown that fetal liver explants in culture provide an excellent model for studying development of hormonal and nutrient control of (a) enzymes of glycogen metabolism and (b) adaptive protein synthesis. With this system it has been possible for the first time to demonstrate a direct effect of insulin on net glycogen synthesis. This effect develops rapidly at birth and is related to increased activation of the enzyme glycogen synthetase by a mechanism requiring translational protein synthesis. Investigating tyrosine aminotransferase induction in this explant system, we found an analogue of bacterial catabolite repression. Glucose inhibits the induction of this enzyme by an amino acid-dependent process. This inhibition by glucose of adaptive protein synthesis is also present in isolated perfused livers from adult rats. Unlike the bacterial system, it does not involve changes in net cyclic AMP turnover.

In vivo and in vitro studies of indole metabolism in the pineal gland have shown that norepinephrine stimulates serotonin N-acetylation by a β-adrenergic-cyclic AMP mechanism. Serotonin and melatonin levels are controlled by the activity of serotonin N-acetyltransferase. This enzyme is induced by a cyclic AMP-mechanism, is inactivated by a photic mechanism, and is stabilized from in vitro inactivation by its substrate, acetyl coenzyme A, or cystamine. The neural pathway regulating N-acetyltransferase activity in the pineal gland includes the recently described retino-hypothalmic projection and the suprachiasmatic nucleus. This nucleus appears to be the "biological clock" which drives circadian rhythms in the pineal enzyme.

The Section on Developmental Pharmacology studies the subcellular processes involved in the induction of pharmacologically important enzymes by drugs, polycyclic hydrocarbons, or insecticides (i.e., xenobiotics). The combination of these environmental stimuli and the host's genetic constitution therefore influence the levels of mono-oxygenase activities in maternal tissues, in the placenta, and transplacentally in fetal tissues. Consequently, we are concerned with (1) the interplay between genetic expression and environmental stimuli, (2) possible effects of xenobiotics on normal fetal growth and development, (3) mechanisms of "pharmacological immaturity" of the premature and newborn, (4) mechanisms of disease of the fetus and newborn involving

oxygenative and conjugative metabolism of normal body substrates such as steroids and bilirubin, and (5) possible therapeutic and diagnostic procedures for aiding and predicting pharmacogenetic defects in the fetus and neonate.

In fetal cell cultures derived from the entire hamster or mouse or from rat liver, we have developed an experimental model having numerous advantages over studies in the whole animal. Thus, we have established a chronological sequence of events occurring during aryl hydrocarbon hydroxylase induction by aromatic hydrocarbons: (1) passive intracellular entry and binding of inducer, (2) synthesis of induction-specific RNA and protein(s), (3) appearance of a spectrally and magnetically distinct high-spin CO-binding hemoprotein associated with hydroxylase activity, and (4) enzymic conversion of inducer to reactive epoxides which bind covalently to cellular macromolecules and which are predominantly excreted by the cell as phenols. With the use of highly inbred mouse strains, we have found that hydroxylase induction in liver, bowel, kidney, lung, and skin in response to systemic aromatic hydrocarbons follows a simple Mendelian autosomal dominant pattern. This dominant expression is furthermore detectable transplacentally in utero and in fetal cell cultures.

In somatic fused-cell hybrids formed between 3T3 mouse fibroblasts in which the hydroxylase is inducible and HTC rat cells in which no basal or inducible hydroxylase is detectable, aryl hydrocarbon hydroxylase induction by aromatic hydrocarbons ranges from the same as, to more than 20-fold greater than, that in the 3T3 parent. Mouse skin tumorigenesis initiated by 7,12-dimethylbenz[a]-anthracene or benzo[a]pyrene and promoted by repeated applications of phorbol ester is unrelated to genetic differences in the extent of hydroxylase induction by polycyclic hydrocarbons in inbred C57BL/6N, DBA/2N, or NZW/BLN or hybrid mice.

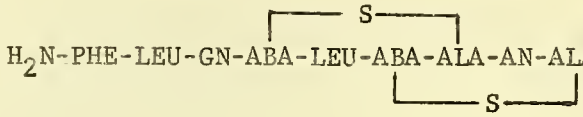
Mono-oxygenase induction in fetal rat hepatocytes and in hepatoma cultures by phenobarbital, p,p'-DDT, or biogenic amines is also under investigation. One action of phenobarbital or biogenic amines, like that of polycyclic hydrocarbons, is at the level of transcription and that with each of the three or more types of inducers there is also another effect at the posttranslational level in which the normal rate of decay of induced enzyme activity is slowed.

The Section on Molecular Structure (1) develops fundamental methods for the structural elucidation and functional correlation of molecules of biological significance and (2) provides advanced approaches to the automation of laboratory processes. The Section isolates and characterizes cell organelles, studies mechanisms of memory and learning, identifies the action of compounds, with preselected structural elements, at the cellular level, and establishes the effect of these compounds on intrauterine development. The chemically oriented activities of the Section supplement other endeavors of the Laboratory as the need arises to determine the structure of constituents of biological systems.

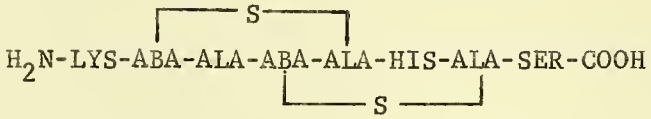
Studies within the Section have progressed in these areas: (1) The structure of nisin has been determined as that of a unique heterodetic pentacyclic peptide. Subtilin has in common with nisin not only the presence of identical numbers of residues of lanthionine, β-methyllanthionine, dehydroalanine, and

β-methyldehydroalanine, but also--at this stage of the structural elucidation--the heterodetic bicyclic system of identical ring sizes of thirteen members each.

In Subtilin:



In Nisin:



Upon exposure to high energy radiation in the presence of tritium, this label entered predominantly into serine and histidine, a fact that may be taken as indication of catalytic (enzyme-like) properties in this relatively small molecule. Photochemical changes in nisin are primarily associated with fragmentations at the double bonds of α,β-unsaturated amino acids. Nisin is capable of inhibiting the synthesis of human corionic gonadotrophin in trophoblast cultures. The presence of lanthionine and β-methyllanthionine in cinnamycin and duramycin led to a search for the occurrence of α,β-unsaturated amino acids in these molecules. The two peptides are free of these amino acids. However, the unsaturations were there at one stage of the biosynthesis. They are now masked by the addition of other amino acids across the double bonds of dehydroalanine and/or β-methyldehydroalanine. The still unknown primary structure of pepsin encouraged us to continue the development of the nonenzymatic cleavage of the aminoacyl bond of S-methylcysteine on pepsinogen. Subsequent to fragmentation at methionine residues, reduction, and methylation of the sulfhydryl groups, one of two residues of S-methylcysteine reacts selectively with cyanogen bromide. (2) The solid phase technique of peptide synthesis in combination with countercurrent distribution lends itself very well to the synthesis of the membrane-active peptides of the gramicidin series. In less than 60 hours, for instance, gramicidin B was synthesized and gave a product that displayed the full biological activity. Employing the same techniques, it was also possible to synthesize the gonadotrophin-releasing factor and variants of the same. (3) Nisin, fragments of nisin, and the gramicidins all display effects on intrauterine development by either inducing fetal resorption or inhibiting implantation, by a mechanism still unknown, when given during early stages of gestation in rodents. (4) The isolation of detergent-free rat liver lysosomes is possible in modest yields, and they are virtually free of plasma membrane contaminants. Lysosomes from human term placenta have been isolated by ultracentrifugation. The combination of several centrifugation techniques promises to provide reasonably pure lysosomes of this poorly understood tissue. (5) Fully automated sample application continued to contribute much to the high performance in our amino acid analytical requirements. A second "home-built" applicator of, in essence, identical design as the first one has just been put in operation.

Serial No. HD-LB1(c)

1. Laboratory of Biomedical Sciences
2. Section on Developmental Enzymology
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Developmental Enzymology

Previous Serial Number: Same

Principal Investigator: J. C. Robinson

Other Investigators: J. B. Edlow
G. Lee

Cooperating Units: P. O. Kohler, Reproduction Research Branch, NICHD

Man Years

Total:	22/12
Professional:	15/12
Others:	7/12

Project Description:

Objectives: The main objectives of this project are: (1) to study qualitative and quantitative changes in selected enzyme systems in the developing placenta and fetal organs in normal gestation; (2) to study qualitative and quantitative changes in these same enzyme systems in the developing placenta and fetal organs in reproductive disorders such as abortions, stillbirths, and fetal malformations; (3) to isolate, purify,and characterize these enzymes from the normal placenta; and (4) to identify and characterize pregnancy-associated enzymes in maternal blood plasma.

Methods Employed: The principal methods employed are: (1) the zymogram technique, by which electrophoretic variants of enzymes can be detected; (2) autoradiography, with which potential carrier function is evaluated (for example, by using I^{131}-tagged thryroxine); (3) enzyme assays, which are usually based on spectrophotometric methods; (4) protein isolation techniques, including salt- and solvent-fractionation, gel-filtration, and ion-exchange chromatography; and (5) immunochemical procedures.

Major Findings: (1) β-Glucuronidase and hexosaminidase have been studied in the JEG-3 clone of choriocarcinoma cells in culture. The isoenzyme patterns and specific activities of these two enzymes are similar to those of normal placenta. However, zymograms of extracts of choriocarcinoma exhibit extra β-glucuronidase and hexosaminidase isoenzymes. Electrophoretic analysis of the medium and its components indicate that the choriocarcinoma cells probably take up the additional isoenzyme from the serum supplements of the culture

medium. Data on hexosaminidase are also obtained which are consistent with the release into the medium of choriocarcinoma-specific hexosaminidase. (2) In term placenta, β-glucuronidase has been partially purified and separated into two major isoenzymic fractions by ammonium sulfate fractionation, a heat step, and a combination of DEAE and CM chromatography. (3) Serum hexosaminidase has also been studied in an effort to determine the source of the elevation of hexosaminidase isoenzyme B in the serum of the pregnant woman. Serum isoenzyme B was not elevated in women treated with estrogen, progesterone, or HCG but was elevated in the serum of women with icteric hepatitis. This is evidence that the elevation of this isoenzyme in gestation is not hormone-mediated by the steroid-producing placenta or ovary; the liver is possibly the source of the gestational increase. (4) Acid phosphatase has also been studied in term placenta and in the choriocarcinoma cells in culture. Although both enzyme preparations show similar Michaelis-Menten constants and V_{max} values, differences exist in the isoenzyme complement of the two types of trophoblast.

Significance to Biomedical Research and the Program of the Institute: The study of placental enzymes in normal pregnancy will provide a suitable baseline with which one can compare the same enzyme systems in placental explants, in benign and malignant trophoblast cells in culture, and in placental tissue from reproductive disorders such as stillbirths and abortions. For example, consistent changes in enzymes in abortion material may reflect differences in placental function, which in turn could affect fetal viability. Hopefully, these studies will lead to increased understanding of both normal and abnormal biochemical processes involved in pregnancy and fetal development.

Proposed Course of Project: (1) Characterization of selected enzymes from placenta, maternal plasma, amniotic fluid, and trophoblast cells in culture. (2) Correlations between abnormal pregnancies and variations in fetal or maternal enzyme systems will be sought.

Honors and Awards: None

Publications:

1. Edlow, J. B., Huddleston, J. F., Lee, G., Peterson, W. F., and Robinson, J. C.: Placental enzymes: specific activities and isoenzyme patterns during early and late gestation. Amer. J. Obstet. Gynec. 111: 360-364, 1971.

2. Edlow, J. B., Huddleston, J. F., Lee, G., Peterson, W. F., and Robinson, J. C.: Multiple enzyme deficiencies in placental tissue from two blighted-ovum abortions. Amer. J. Obstet. Gynec. 111: 364-368, 1971.

3. Huddleston, J. F., Cefalo, R. C., Lee, G., and Robinson, J. C.: An investigation of the gestational increase in serum hexosaminidase B. Amer. J. Obstet. Gynec. 111: 804-807, 1971.

Serial No. [illegible] LP?
1. Laboratory of Biomedical Sciences
2. Section on Intermediary Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Intracellular Proteolytic Enzymes

Previous Serial Number: Same

Principal Investigator: G. Guroff

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1/12
Professional:	1/12
Others:	0

Project Description:

Objectives: The objectives of this research are: (1) to purify and characterize specific intracellular proteolytic enzymes; (2) to determine the function of these enzymes; and (3) to investigate how these enzymes are linked into the overall metabolism of the cell.

Methods Employed: The methods used include standard techniques of protein purification and assay, fluorometric analysis, and antibody preparation.

Major Findings: (1) Partial purification of an inhibitor for an intracellular proteinase from brain; (2) preparation of animals immunized against the inhibitor.

Significance to Biomedical Research and the Program of the Institute: Although intracellular proteolytic enzymes have been recognized for many years, their function is unknown. Assignment of a mechanism of action, physiological or enzymatic, to one of these enzymes would add significantly to biochemical information. Such information might also be useful therapeutically.

Proposed Course of Project: (1) Complete purification of and characterization of the inhibitor; (2) further purification of the enzyme; (3) studies on the mode of action of both enzyme and inhibitor.

Honors and Awards: None

Publications: None

Serial No. HD-LB9
1. Laboratory of Biomedical Sciences
2. Section on Intermediary Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Models of Mental Retardation

Previous Serial Number: Same

Principal Investigator: G. Guroff

Other Investigators: A. Andersen
A. Abramowitz
D. Rudd

Cooperating Units: None

Man Years

Total:	24/12
Professional:	21/12
Others:	3/12

Project Description:

Objectives: The objectives of this research are: (1) to produce biochemical models of mental retardation syndromes in laboratory animals by chemical means; (2) to investigate the pathological and behavioral aspects of the chemical treatments; (3) to study the mechanisms by which specific chemical alterations produce a change in normal neural functioning; (4) to attempt new methods for preventing such alterations hopefully pointing the way for new clinical approaches.

Methods Employed: The methods employed include standard enzymatic assay techniques, histological examinations, and behavioral evaluation.

Major Findings: (1) Treatment of newborn rats with p-chlorophenylalanine plus phenylalanine produced a biochemical picture similar to that seen in clinical phenylketonuria, e.g., high blood phenylalanine-to-tyrosine ratio, phenylketones in the urine, low phenylalanine hydroxylase levels. (2) Such treatment during the development of the rat (0-21 days) produces, in the adult animal, behavioral and pathological alterations analogous to clinical phenylketonuria, e.g., hyperactivity, deficiencies in problem solving, smaller brains.

Significance to Biomedical Research and the Program of the Institute: No adequate model of mental retardation has yet been produced in a laboratory animal. The need for such a disease analog is great, since only then can

investigations be initiated toward understanding the biochemical basis for the retardation. The endeavor to produce a chemical model in this Laboratory centers around phenylketonuria because it is the best characterized from a biochemical and a behavioral aspect and because a great deal is known about the enzyme which is deleted, phenylalanine hydroxylase. If such a model can be produced, substantial information should be available about the causes and perhaps the prevention of the retardation.

Proposed Course of Project: (1) A complete biochemical profile of the effects of p-chlorophenylalanine, a potent phenylalanine hydroxylase inhibitor; (2) studies on its mode of inhibition; (3) administration of this drug to rats during development and pathological and behavioral evaluation after growth to maturity; (4) attempts to prevent the defect from developing by simultaneous treatment with other materials; (5) studies of the appearance of associated brain lesions; (6) expansion of this approach to a consideration of other metabolic abnormalities leading to retardation.

Honors and Awards: None

Publications:

1. Andersen, A., and Guroff, G.: Enduring behavioral changes in rats with experimental phenylketonuria. Proc. Nat. Acad. Sci., U.S.A., in press.

2. Andersen, A., Abramowitz, A., and Guroff, G.: Biochemical and behavioral changes in rats with experimental phenylketonuria. In Barchas, J. (Ed.): Behavioral Effects of Changes in Level of Brain Serotonin. New York, Academic Press, in press.

Serial No. HD-LB10
1. Laboratory of Biomedical Sciences
2. Section on Intermediary Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mechanism of Aromatic Hydroxylation

Previous Serial Number: Same

Principal Investigator: G. Guroff

Other Investigators: G. Dickens
A. Abramowitz
K. Bromwell

Cooperating Units: W. Lovenberg, Experimental Therapeutics Branch, NHLI
R. Bensinger, Experimental Therapeutics Branch, NHLI
H. Yamabe, Experimental Therapeutics Branch, NHLI

Man Years

Total:	30/12
Professional:	9/12
Others:	21/12

Project Description:

Objectives: The objectives of this research are: (1) to provide information on the process of enzymatic hydroxylation; and (2) to get detailed information on the nature of phenylalanine hydroxylase.

Methods Employed: The methods employed include standard techniques of enzyme kinetics and purification, antibody detection, and organic synthesis and characterization.

Major Findings: (1) Various hydroxylase enzymes exhibit quite different cofactor specificities. (2) Aquayamycin, a new, hydroxylase-specific antibiotic, inhibits bacterial and liver phenylalanine hydroxylases in an irreversible, sulfhydryl-dependent manner. (3) RNA's of all kinds strongly inhibit liver phenylalanine hydroxylase but not bacterial phenylalanine hydroxylase. (4) p-Chlorophenylalanine, an inhibitor of phenylalanine and tryptophan hydroxylases in the rat, is activated to a very small extent by E. coli and rat liver t-RNA-activating enzymes. (5) Comamonas sp., a bacterium which hydroxylates phenylalanine as do mammals, also metabolizes tyrosine using a degradative pathway similar to that found in mammals.

Significance to Biomedical Research and the Program of the Institute: Information about phenylalanine hydroxylase and about ways to inhibit or

stimulate it relates directly to the problem of mental retardation since the enzyme in question is the one missing in the best characterized of the genetic diseases, phenylketonuria. Detailed analysis of the structure of this enzyme should provide new and unique information since very little has been accomplished on hydroxylases in general and no pteridine enzymes have been reported as homogeneous preparations.

Proposed Course of Project: (1) Continued study of the mode of action and interaction of phenylalanine hydroxylase; (2) survey of other phenylalanine hydroxylating organisms to find sources of the hydroxylase enzyme which may not be so resistant to purification as Comamonas sp.

Honors and Awards: None

Publications: None

Serial No. HD-LB11

1. Laboratory of Biomedical Sciences
2. Section on Intermediary Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Biosynthesis and Function of Pteridines

Previous Serial Number: Same

Principal Investigator: G. Guroff

Other Investigators: J. Cone
J. Dayman

Cooperating Units: None

Man Years

Total:	14/12
Professional:	5/12
Others:	9/12

Project Description:

Objectives: The objectives of this research are: (1) to provide information on the enzymes in the biosynthetic pathway to the pteridine ring; (2) to investigate the functions of pteridines in addition to their action as hydroxylase cofactors.

Methods Employed: The methods used include standard techniques of enzyme purification, isotope measurement, high-voltage electrophoresis, and fluorescence detection.

Major Findings: The discovery that two enzymes in Comamonas sp. catalyze the first step in pteridine biosynthesis was made. The second enzyme has properties different from the first enzyme found, e.g., chromatographic behavior and ionic requirements, and it probably has a different metabolic function, even though the reaction catalyzed seems superficially to be the same.

Significance to Biomedical Research and the Program of the Institute: The pteridine cofactors are important in a number of hydroxylase reactions including those leading to the neurohormones serotonin and norepinephrine. Information about the pharmacology and biochemistry of these biosynthetic enzymes may lead to the ability to inhibit or stimulate these pathways and perhaps to new therapeutic tools for maladies involving these neurohormones.

Proposed Course of Project: (1) Purification of the two GTP cyclohydrolases from Comamonas sp.; (2) comparison of properties; (3) assignment of metabolic

function to each; (4) purification and characterization of other enzymes of pteridine metabolism; (5) attempts to link pteridines or pteridine derivatives to the enzymes they serve; (6) study of pteridine biosynthetic enzymes in photosynthetic organisms; (7) search for other functions of pteridines.

Honors and Awards: None

Publications: None

Serial No. HD-LB12
1. Laboratory of Biomedical Sciences
2. Section on Intermediary Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Biochemistry of Brain Macromolecules

Previous Serial Number: Same

Principal Investigator: G. Guroff

Other Investigators: M. Brodsky
W. Gutensohn
J. DeLarco
S. Nakagawa

Cooperating Units: None

Man Years

Total:	38/12
Professional:	35/12
Others:	3/12

Project Description

Objectives: The objectives of this research are: (1) to investigate the biochemistry of brain macromolecules; (2) to determine if any aspects exist that are unique to brain; and (3) to find out whether any unique aspects are related to the unique function of the brain.

Methods Employed: The methods involved in this study include isotope techniques, high voltage electrophoresis, and tissue slice metabolic studies, as well as standard methods for purification and investigation of macromolecules.

Major Findings: (1) Guanine-hypoxanthine:PRPP pyrophosphorylase from rat brain has been studied biochemically, immunologically, developmentally, and pharmacologically. A new isotopic method for assay has been developed. (2) The transport of purine bases into brain has been investigated and some interesting details of metabolism have been worked out. (3) The synthesis of RNA in rat brain has been studied in a slice system. The presumptive synthesis of messenger RNA has been observed and attempts are being made to use the brain messenger to prime the cell-free synthesis of brain-specific proteins.

Significance to Biomedical Research and the Program of the Institute: Substantial evidence exists that brain macromolecules are involved in the unique functions of the brain. A continuing question exists as to the

directness of this involvement. These studies may shed light on this question by finding properties of brain macromolecules compatible with a unique function such as the storage of memory. Further, development of such systems as the tissue slice for RNA synthesis may provide tools for the investigation of the biochemical alterations occurring in certain types of mental retardation.

Proposed Course of Project: (1) Continued investigation of the nature and function of the purine:PRPP pyrophosphorylases of brain; (2) attempts to inhibit these enzymes chemically and study the behavior consequences, e.g., as a laboratory model of the Lesch-Nyhan syndrome; (3) investigation of the transport system for purines in brain; (4) quantitative and qualitative characterization of rat brain messenger RNA; (5) use of messenger in a cell-free protein synthesizing system.

Honors and Awards: None

Publications:

1. Hogans, A. F., Guroff, G., and Udenfriend, S.: Studies on the origin of pyrimidines for neural RNA biosynthesis in the rat. J. Neurochem. 18: 1699-1710, 1971.

2. Guroff, G., and Brodsky, M.: Enzymes of nucleic acid metabolism in the brains of young and adult rats. J. Neurochem. 18: 2077-2084, 1971.

3. Guroff, G.: Biochemistry of the brain. In Mason, H. S. (Ed.): Functional Biochemistry. MacMillan Co., in press.

4. Guroff, G.: Special aspects of amino acid metabolism. In Siegel, G. (Ed.): Basic Neurochemistry. Little, Brown and Co., in press.

5. Gutensohn, W., and Guroff, G.: A rapid assay purine phosphoribosyl transferases. Anal. Biochem., in press.

6. Gutensohn, W., and Guroff, G.: Hypoxanthine-guanine phosphoribosyltransferase from rat brain (purification, kinetic properties, development, and distribution). J. Neurochem., in press.

Serial No. HD-LB13

1. Laboratory of Biomedical Sciences
2. Section on Intermediary Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mechanism of Amino Acid Transport

Previous Serial Number: Same

Principal Investigator: G. Guroff

Other Investigators: K. Bromwell

Cooperating Units: None

Man Years

Total:	7/12
Professional:	1/12
Others:	6/12

Project Description:

Objectives: The objectives of this research are: (1) to describe the process by which amino acids enter cells; (2) to investigate biochemical and pharmacological effects on the transport system; and (3) to dissect the transport system.

Methods Employed: The methods employed include standard protein purification, isotope techniques, and bacterial culture operations.

Major Findings: (1) Complete purification and partial characterization of a phenylalanine-binding protein from transport-induced bacteria; (2) partial purification of two other proteins from the periplasmic envelope of Comamonas and characterization of one as a copper-containing protein.

Significance to Biomedical Research and the Program of the Institute: The active transport of amino acids is a general property of cells. The transport of amino acids into brain or kidney, etc., may be a limiting or at least a contributory factor in their further metabolism. An understanding of the mechanisms of transport may provide insight into the normal functioning of cells and into ways to cope with transport malfunctions which occur in certain disease states.

Proposed Course of Project: (1) Investigation of methods for large scale preparation of the phenylalanine-binding protein and a study of its chemical structure; (2) further study of the copper-binding protein from Comamonas; (3) search for comparable sites in transport systems of mammalian tissues.

Honors and Awards: None

Publications:

1. Kuzuya, H., Bromwell, K., and Guroff, G.: The phenylalanine-binding protein of Comamonas sp. (ATCC 11299a). J. Biol. Chem. 246: 6371-6380, 1971.

Serial No. HD-LB4
1. Laboratory of Biomedical Sciences
2. Section on Physiological Controls
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Regulation of Liver Metabolism

Previous Serial Number: Same

Principal Investigator: W. H. Glinsmann

Other Investigators: P. Sherline
H. Eisen

Cooperating Units: I. Goldfine, Clinical Endocrinology Branch, NIAMD

May Years

Total:	54/12
Professional:	30/12
Others:	24/12

Project Description:

Objectives: This project seeks to define in isolated mammalian liver systems: (1) mechanisms underlying differential modulation of glucose metabolism and adaptive protein synthesis by hormones, nutrients, and biogenic amines; (2) factors controlling the development of these mechanisms. Current model systems concentrate on interactions between: (1) hormones and biogenic amines which alter the production or action of adenosine 3':5'-monophosphate, (2) glucose, and (3) amino acids.

Methods Employed: Isolated rat liver systems (cells, explants in culture, isolated perfused livers) are used in studying control of liver glucose output, and regulatory enzymes in glycogen metabolism, gluconeogenesis, and amino acid catabolism. In conjunction with physiological and pharmacological experiments in these systems, assays are performed for metabolic intermediates and enzyme activities in incubation media, tissue homogenates, and subcellular or protein fractions.

Major Findings: 1. Development of hormonal and substrate regulation of glycogen turnover in isolated fetal and neonatal liver: Conditions have been established for maintaining in defined media viable, hormonally-responsive explants of neonatal rat liver. Using this system, we have confirmed in vivo observations which show development of glycogen synthesis and the appearance of phosphorylase and glycogen synthetase during the last 4 days of gestation. Development of the adenyl cyclase system, which is known to occur at the same time, correlates with the development of an effect of glucagon or epinephrine

to increase tissue levels of cyclic AMP and to activate phosphorylase. Acute regulation of phosphorylase activity by insulin is similar to that which occurs in isolated perfused adult rat liver. Insulin has no effect by itself but inhibits phosphorylase activation by epinephrine. Regulation of glycogen synthetase activity by glucose, an effect in adult liver which contributes to autoregulation of hepatic glucose output, is not present in explants of term fetal liver.

The most significant finding with this system is that insulin induces over 4 to 6 hours glycogen synthesis and the conversion of glycogen synthetase to an active form. This effect is inhibited by cycloheximide but not actinomycin D supporting the possibility that this action of insulin requires translational protein synthesis. This is the first demonstration of a direct effect of insulin on net glycogen synthesis in isolated mammalian liver tissue. This action of insulin is not associated with induction of glucokinase; it is not altered by glucocorticoids; and it rapidly increases in magniture during the first day of postnatal life.

2. Regulation of rat liver glycogen synthetase and phosphorylase activities by glucose and cyclic AMP: Cyclic AMP activates a protein kinase which phosphorylates glycogen synthetase and phosphorylase; glucose given to isolated adult livers causes a dephosphorylation of these enzymes by a mechanism which is independent of changes in cyclic AMP metabolism. We have continued to examine possible sites of glucose action in controlling enzyme reactions using purified or partially purified enzyme preparations. Using partially purified (150-300 fold) glycogen synthetase as substrate for purified cyclic AMP-dependent protein kinase, we could not show that glucose or glucose metabolites altered the kinetic behavior or cyclic AMP-binding to the protein kinase. To date, attempts at purification of glycogen synthetase phosphatase have been unsuccessful.

Control by epinephrine of cyclic AMP synthesis in isolated perfused rat liver appears to involve both α and β type receptors; the β type stimulates cyclic AMP formation, and the α type inhibits β stimulation of cyclic AMP synthesis. Control of phosphorylase activation by epinephrine involves both α (cyclic AMP-independent and β (cyclic AMP-mediated) receptor function. Most likely the α-receptor effect is mediated by alterations in vascular tone and direct effects of epinephrine on the hepatocyte are mediated by a β-receptor. Adrenergic control of phosphorylase activation in isolated nonvascular explants of early postnatal liver is inhibited only by β antagonists.

3. Glucose repression of tyrosine aminotransferase synthesis. Bacterial catabolite repression (glucose repression) of substrate-induced enzyme synthesis is regulated by intracellular cyclic AMP levels. A similar phenomenon of glucose repression has been shown to exist in rat liver. In isolated livers from fasted rats, amino acid loading induces a rapid synthesis of tyrosine aminotransferase, and hyperglycemia inhibits this induction. Unlike the bacterial system, no concomitant alterations in cyclic AMP metabolism has been detected. Glucose was also not effective in inhibiting enzyme induction by glucagon cyclic AMP, insulin, or hydrocortisone. The effect of glucose in inhibiting amino acid induction of tyrosine transaminase could be separated from the action of glucose in activating glycogen synthetase. This

was demonstrated by the use of livers from adrenalectomized-fasted rats. These livers, deficient in synthetase phosphatase and diabetic-like in their response to glucose, are responsive to glucose repression.

It has been reported that a spontaneous rise in tyrosine aminotransferase activity occurs in late fetal explants in culture. This effect has been verified. It is dependent on amino acids, requires continuing protein synthesis, and is subject to glucose repression. Induction of enzyme synthesis by cyclic AMP or hydrocortisone in the presence of a complete mixture of amino acids is not altered by glucose. High medium glucose, however, does inhibit tyrosine aminotransferase synthesis by these agents when amino acid-deficient media is used. This lends support to the idea that glucose may be interfering with amino acid metabolism. No single amino acid induces enzyme synthesis or fully substitutes for a complete mixture which supports maximal rates of enzyme synthesis in response to hydrocortisone.

4. Gluconeogenesis-induction of phosphoenolpyruvate carboxykinase. This enzyme which appears to be rate limiting in the conversion of lactate or pyruvate to glucose is known to be induced at birth and by fasting by a cyclic AMP-mediated mechanism. An extremely sensitive assay for this enzyme has been developed by Walsh. Modifying this basic assay, we have shown the liver's supernatant enzyme has 2 pH optima and 2 apparent K_m values for oxaloacetic acid. A low K_m ($3\text{-}4\times10^{-6}$M) form is present in livers from fed and fasted rats; fasting induces a high-K_m (3×10^{-5}M) form. Possible mechanisms for activation of this high-K_m enzyme are being investigated.

Significance to Biomedical Research and the Program of the Institute: Regulation of cellular metabolism, growth, and differentiation at many phylogenetic levels appears to be mediated by a complex series of interactions between glucose and nutrients and factors which alter the net production or cellular levels of cyclic AMP. In mammalian systems, many polypeptide hormones and biogenic amines act primarily by altering the formation of this nucleotide. The present study, defining mechanisms by which preexisting nutritional state and acute alterations in nutrient supply modify these hormonal responses in liver, will hopefully increase our understanding of factors which regulate the development and adaptations of hepatic enzymes and control glucose homeostasis.

Proposed Course of Project: Studies will continue to examine mechanisms which control cyclic nucleotide, protein, and carbohydrate metabolism in liver.

The mechanism whereby insulin alters liver glycogen synthetase will be explored as will its possible relationship to the development of glucose autoregulation. Factors controlling the development of this action of insulin will hopefully increase our knowledge about intracellular mechanisms mediating the action of this hormone.

Studies on glucose repression of adaptive enzyme synthesis will be conducted to see if this effect contributes to the developmental or adaptive regulation of other regulatory enzymes in carbohydrate and amino acid metabolism which are known to be induced by cyclic AMP or glucocorticoids and are repressed

by feeding. Possible controlling sites of this effect of glucose will be examined.

Control of phosphoenolpyruvate carboxykinase synthesis and potential activating mechanisms will be studied in fetal and adult tissue. Development of this enzyme, which has been postulated to be actively regulated by cyclic AMP and insulin, correlates with the development of gluconeogenesis.

Honors and Awards: None

Publications:

1. Sherline, P., Lynch, A., and Glinsmann, W.: Cyclic AMP and adrenergic receptor control of rat liver glycogen phosphorylase activity. Endocrinology, in press.

Serial No. HD-LB18
1. Laboratory of Biomedical Sciences
2. Section on Physiological Controls
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Regulation of Neuroendocrine Metabolism

Previous Serial Number: Same

Principal Investigator: D. C. Klein

Other Investigators: S. A. Binkley
M. A. Vaughan

Cooperating Units: R. J. Reiter, Department of Anatomy, University of Texas Medical School, San Antonio, Texas
R. E. Moore, Division of Neurology, University of Chicago, Chicago, Illinois
C. L. Ralph, Department of Biology, University of Pittsburgh, Pittsburgh, Pennsylvania
R. Bensinger and W. Lovenberg, Experimental Therapeutics Branch, NHLI
B. Hoffer and F. Bloom, Laboratory of Neuropharmacology, Devision of Special Mental Health Research, NIMH
G. Brownstein and G. Ross, Reproduction Research Branch, NICHD
A. Yuwiler, Neurobiochemistry Laboratory, V.A. Brentwood Hospital, Los Angeles, California

Man Years

Total:	60/12
Professional:	42/12
Others:	18/12

Project Description:

Objectives: (1) To describe the mechanisms through which neuronal activity regulates biochemical activity in a neuroendocrine tissue, the pineal gland, and in the brain; (2) to describe factors regulating the development of enzyme systems and regulatory mechanisms in neural and neuroendocrine tissues; (3) to describe the determining factors which direct the innervation of neuroendocrine tissue by nerve fibers from a sympathetic ganglion; (4) to describe the role of N-acetylation of serotonin in the brain; (5) to describe the physiology of melatonin; (6) to isolate and characterize pineal peptides which influence reproductive physiology.

Methods Employed: The most sensitive biochemical indication of neuronal effects is pineal N-acetyltransferase activity. This is measured in an *in vitro* system; the effects of neurochemicals and hormones are determined. In addition, the product of this enzyme, N-acetylserotonin, and the precursor, serotonin, are studied. Pharmacological agents are used as tools to reveal the physiological mechanisms involved in the regulation of the enzyme and associated compounds. The neural pathway which carries visual signals through the central nervous system by way of the autonomic nervous system to the pineal gland is studied by making lesions in the brain and determining if isolation or destruction of a specific area distrubs the normal 15-70 fold circadian rhythm in the enzyme.

The *in vitro* system is also used to study the development of enzyme systems in the pineal gland. Compounds that might induce the appearance of a specific enzyme are tested for ability to stimulate the appearance of that enzyme in neonatal tissue.

The factors regulating the innervation of the pineal gland are studied by examining the effect of pineal extracts and pineal secretions on the outgrowth of nerve fibers from superior cervical sympathetic ganglia grown in culture.

The physiology of N-acetylated derivatives of serotonin is studied by measuring the concentrations in tissues and body fluids and the distribution of the enzymes which make and destroy these compounds.

The isolation and characterization of pineal peptides which influence reproductive physiology is pursued by purifying these compounds from beef pineal glands and testing their biological effect in a bioassay.

Major Findings: 1. Neural regulation of biochemical activity: We have developed an analytical method which makes it possible to detect N-acetylserotonin in pineal glands. Using this new method it has been found that the activity of N-acetyltransferase is directly correlated with the concentration of N-acetylserotonin *in vivo* and *in vitro*. The production of N-acetylserotonin *in vitro* is also directly correlated with enzyme activity. Measurement of the concentration of serotonin in cultured pineal glands has shown that it exhibits an inverse relationship to the activity of N-acetyltransferase. Pharmacological studies have indicated that norepinephrine regulates these changes. The initial effect is an increase in the activity of adenyl cyclase which is associated with a rapid increase in the concentration of cyclic AMP in pineal cells as indicated by histochemical and biochemical methods. The effects of norepinephrine on adenyl cyclase are via a highly specific β-adrenergic receptor. Cyclic AMP decreases serotonin and increases N-acetylserotonin by increasing the activity of N-acetyltransferase. The increase in intracellular N-acetylserotonin causes a mass-action increase in the production of melatonin and an increased release of N-acetylserotonin and melatonin.

The activity of N-acetyltransferase is 15-70 fold higher at night. The activity decreases with a half life of 3 minutes when animals are exposed to light at night. A similar rapid decrease occurs if "night" pineals are homogenized and incubated at 37°. This disappearance is prevented if the homogenates are incubated with the cofactor of the reaction, acetyl coenzyme A.

A small fragment of acetyl coenzyme A, cystamine, which is the mercapto-terminal portion can prevent the degradation almost as well as can acetyl coenzyme A.

The neural pathway regulating N-acetyltransferase activity in the pineal gland includes the recently described retino-hypothalamic projection and the suprachiasmatic nucleus, which is located in the anterior hypothalamus. The results of these studies indicate that this nucleus may be the site of a biological clock which drives circadian rhythms in pineal N-acetyltransferase and adrenal corticoid secretion. Light from the eye appears to suppress the generation of stimulatory signals from this nucleus to the pineal gland, by way of fibers in the medial forebrain bundle. This research has also revealed that there are probably two neural pathways through the central nervous system via the superior cervical ganglia to the pineal gland. One mediates photic effects on N-acetyltransferase and the other regulates hydroxyindole-O-methyl-transferase.

We have found it is possible to study the hydroxylation of tryptophan, a precursor of serotonin, in intact tissues cultured for several days. This allows the examination of the mechanisms regulating this reaction in an experimental condition that stimulates the physiological environment of the tissue. It appears that hydroxylation does not change appreciably in chronically denervated glands, nor is there a sex difference in tryptophan hydroxylation. Norepinephrine appears to increase total hydroxylation by increasing the concentration of substrate.

2. Pineal peptides: Several pineal peptides can alter reproductive function. There is one and pherhaps more peptides that appear to inhibit gonadal function and one peptide that enhances gonadal function. These peptides are below m.w. = 3000 and are soluble in organic solvents. They are being prepared for characterization.

We have been able to detect the presence of anti- and pro-gonadotrophic peptides in 1-10% of a rat pineal gland, and in the culture medium of pineal organ cultures.

Significance to Biomedical Research and the Program of the Institute: These investigations have provided a description of a complex mechanism through which rapid neurally mediated changes in the biochemistry of cells can occur. This establishes a model of how one neurotransmitter, norepinephrine, can regulate the metabolism of another, serotonin, through a highly specific, noncompetitive mechanism. This efficient control process might be repeated wherever there is interaction between adrenergic nerve endings and serotonergic cells, such as in the raphé nuclei, and in the hypothalamus. The pineal may provide a model that has general distribution in the brain and which might underlie rapid mental process and changes in behavioral states. This is especially exciting in view of the evidence indicating that serotonin in the raphé nuclei might be involved in controlling sleep.

These studies may have uncovered an important "biological clock" which regulates circadian rhythms. The close proximity of this area, the supra-chiasmatic nucleus, to the hypothalamus and the known connection between the

suprachasmatic nucleus and the hypothalamus raise the possibility that this site has general control over circadian rhythms.

The antigonadotrophic potential of the pineal gland does not seem to lie primarily in melatonin or any other pineal indole. It may lie in pineal peptides. These peptides may provide valuable therapeutic and diagnostic tools in reproductive endocrinology. In addition they may play a role in pathological disturbances in reproductive physiology that appear to be mediated by the pineal gland.

Proposed Course of Project: The study of the regulation of N-acetyltransferase will be focused on the intracellular mechanisms regulating the rapid changes in enzyme activity and also the mechanisms involved in the development of the regulatory processes. The possible role of cysteamine as an intracellular regulator or intracellular messenger will be explored. The function of N-acetylation of serotonin in the brain and the regulation of N-acetylation of serotonin will be investigated. N-acetylserotonin in the cerebral spinal fluid will be measured and correlated with mental states and physiological processes. It is hoped that the functional significance of N-acetylation of serotonin in the brain will become evident from these studies. It is also anticipated that the description of the rapid regulation of N-acetyltransferase in the pineal gland will provide a model of how the activity of other enzymes might be regulated by neuronal activity.

The pineal peptides will be isolated, their amino acid composition and amino acid sequence will be determined. The physiological effects of these peptides will be characterized and it is hoped that synthetic peptides might be made that would be used in a broad study investigating the possible therapeutic or diagnostic use of the pineal peptides in reproductive endocrinology.

The radioimmunoassay of melatonin will be developed and applied to studies of the physiology of melatonin in humans and experimental animals. In addition to a possible role in reproductive physiology, a potential role of melatonin in behavior will be investigated.

Honors and Awards: None

Publications:

1. Reiter, R. J., and Klein, D. C.: Observations on the pineal gland, the Harderian glands, the retina, and the reproductive organs of adult female rats exposed to continuous light. J. Endocr. 51: 117-125, 1971.

2. Klein, D. C., Reiter, R. J., and Weller, J. L.: Pineal N-acetyl-transferase activity in blinded and anosmic male rats. Endocrinology 89: 1020-1023, 1971.

3. Berg, G. R., and Klein, D. C.: Pineal gland in organ culture. II. Role of adenosine 3',5'-monophosphate in the regulation of radiolabeled melatonin production. Endocrinology 89: 453-464, 1971.

4. Klein, D. C. Weller, J. L., and Moore, R. Y.: Melatonin metabolism: neural regulation of pineal serotonin: acetyl coenzyme A N-acetyltransferase activity. Proc. Nat. Acad. Sci., U.S.A. 68: 3107-3110, 1971.

5. Lott, I. T., Quarles, R. H., and Klein, D. C.: Biosynthesis and release of glycoproteins by pineal gland in organ culture. Biochim. Biophys. Acta 264: 144-151, 1972.

6. Berg, G. R., and Klein, D. C.: Norepinephrine stimulates ^{32}P incorporation into phosphatidyl inositol in membranes of cultured pineal glands. J. Neurochem., in press.

7. Ellison, N., Weller, J., and Klein, D. C.: Development of a circadian rhythm in the activity of pineal serotonin N-acetyltransferase. J. Neurochem., in press.

8. Silberstein, S. D., Lemberger, L., Klein, D. C., Axelrod, J., and Kopin, I. J.: Induction of adrenal tyrosine hydroxylase activity in organ culture. Neuropharmacology, in press.

9. Klein, D. C.: Evidence for the placental transfer of melatonin [acetyl-^{3}H]. Nature, in press.

10. Strada, S. J., Klein, D. C., Weller, J., and Weiss, B.: Effect of norepinephrine on the concentration of adenosine 3',5'-monophosphate of rat pineal gland in organ culture. Endocrinology, in press.

11. Klein, D. C.: The role of serotonin N-acetyltransferase in the adrenergic regulation of indole metabolism in the pineal gland. In Usdin, E., and Barchas, J. (Eds.): Serotonin and Behavior. New York, Academic Press, in press.

12. Klein, D. C.: Regulation of melatonin metabolism. In Kopin, I. (Ed.): Methods in Investigative and Diagnostic Endocrinology. Amsterdam, North-Holland Publishing Co., in press.

Serial No. HD-LB8(1)
1. Laboratory of Biomedical Sciences
2. Section on Developmental Pharmacology
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Developmental Pharmacology

Previous Serial Number: Same

Principal Investigator: D. W. Nebert

Other Investigators: J. E. Gielen
F. E. Goujon
W. F. Benedict
I. S. Owens
N. Considine

Cooperating Units: R. M. Friedman, Laboratory of Pathology, NCI
E. B. Thompson, Laboratory of Biochemistry, NCI
J. W. Daly, Laboratory of Chemistry, NIAMD

Man Years

Total:	57/12
Professional:	44/12
Others:	13/12

Project Description:

Objectives: Membrane-bound mixed-function oxygenases are important in the metabolism of drugs, carcinogens, and insecticides, as well as many endogenous lipophilic substrates such as steroids, hemin, and bilirubin. We found that one such oxygenase, aryl hydrocarbon hydroxylase, is inducible in mammalian fetal liver cells grown in culture by polycyclic hydrocarbons and by phenobarbital. Whereas it is well known that polycyclic hydrocarbons and phenobarbital induce hepatic drug-metabolizing enzymes by different mechanisms, the precise effects evoked by these two classes of microsomal enzyme inducers are not understood. Cell culture thus provides an opportunity for studying the mechanisms of microsomal oxygenase induction in an experimental system which is more rigidly controlled than similar studies in the intact animal. Understanding the mechanisms by which drug-metabolizing enzymes are induced may elucidate important concepts in drug metabolism, developmental pharmacology, steroid biochemistry, chemical carcinogenesis, and entomology. The main objectives of this project are: (a) to compare the rates of hydroxylase synthesis and degradation and the physical properties of the phenobarbital-induced enzyme with those of the hydroxylase induced by polycyclic hydrocarbons, (b) to determine the extent to which hormones and nutrition affect the hydroxylase induction by either compound, (c) to characterize specific

changes in nuclear acidic proteins, cellular RNA, and microsomal membrane protein during the induction process by either compound, (d) to measure entry and binding of various inducers to specific receptor sites within the hepatocytes, (e) to develop methods for inhibiting or stimulating the sequence of events involved during the oxygenase induction by either class of inducers, and (f) to use this experimental system for studying the control of specific gene expression in higher organisms.

Methods Employed: The principal methods employed are: (1) recording spectrophotometry of turbid solutions, (2) enzyme assay involving spectrophotofluorometric determination of product formation, (3) radioisotopic measurements using tritium and carbon-14, (4) cell culture techniques, (5) differential centrifugation, (6) phase microscopy, (7) equilibrium dialysis, (8) polyacrylamide gel electrophoresis, (9) column chromatography, and (10) magnetic resonance techniques.

Major Findings: (1) Aryl hydrocarbon hydroxylase activity is inducible in rat fetal hepatocytes in culture by phenobarbital, the insecticide 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane, benz[a]anthracene, or 3-methylcholanthrene. (2) Between the second and fourth day of age of the primary cultures, the maximal net rate at which the hydroxylase activity accumulates is about the same when phenobarbital, benz[a]anthracene, or 3-methylcholanthrene is in the growth medium at optimal concentrations. (3) The optimal inducing concentrations for each compound are 2.0 mM phenobarbital, 100 μM 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane, 13 μM benz[a]anthracene, and 0.75 μM 3-methylcholanthrene. (4) The kinetics of the oxygenase induction and degradation are the same in liver cell cultures exposed to either phenobarbital or benz[a]anthracene; the time required for doubling the hydroxylase activity is about 3.0 hours, and the initial half-life of the induced enzyme system is about 10.5 hours. (5) An additive effect is obtained when either phenobarbital or 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane is present with a polycyclic hydrocarbon in the medium but not when the hepatocytes are treated with phenobarbital plus 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane or with the combination of benz[a]anthracene and 3-methylcholanthrene. (6) The apparent Michaelis constants for the control hydroxylase and for the enzyme system inducible by either phenobarbital or benz[a]anthracene range between 2 and 4 μM in the presence of the substrate benzo[a]pyrene. (7) Compared with the induction process by benz[a]anthracene, aryl hydrocarbon hydroxylase induction in fetal rat hepatocyte cultures by phenobarbital is more sensitive to low doses of either actinomycin D or cycloheximide; by extrapolation to other studies *in vivo* and in cell culture, nucleolar synthesis of ribosomal RNA may be of more importance in the process of microsomal enzyme induction by phenobarbital than that by benz[a]anthracene. (8) Degradation of the phenobarbital- or benz[a]anthracene-induced hydroxylase activity is the same whether the enzyme system is maximally or half-maximally induced: the half-life is 10.5 ± 3.6 hours in fresh control medium and 8.5 ± 2.5 hours in the presence of high levels of cycloheximide. (9) Actinomycin D stabilizes or slightly stimulates the enzyme activity in fetal liver cells previously treated with either phenobarbital of benz[a]anthracene. (10) The continued presence of either inducer in hepatocytes exposed to high levels of cycloheximide or actinomycin D also stabilizes the phenobarbital- or benz[a]-anthracene-induced hydroxylase activity. (11) Actinomycin D does not prevent

the induction of oxidase activity in cells previously treated with cycloheximide and either inducer. (12) One action of phenobarbital, like that of benz[a]anthracene, is at the level of transcription; with either inducer there is also another effect at the posttranslational level in which the normal rate of decay of the induced enzyme is delayed. (13) In the inbred C57BL/6N, C3H/HeN, AL/N, or C3Hf/HeN mouse, aryl hydrocarbon hydroxylase induction by aromatic hydrocarbons increases rapidly in the neonatal period; this response to these xenobiotics does not appear in the DBA/2N, NZW/BLN, NZB/BLN, or AKR/N strains. (14) The hydroxylase induction by aromatic hydrocarbons is inherited as a simple autosomal dominant trait, designated the ah locus. (15) Induction of mono-oxygenase activity by phenobarbital does not follow a simple Mendelian pattern. (16) The formation of a spectrally and magnetically distinct carbon monoxide-binding cytochrome from liver microsomes is associated with the polycyclic hydrocarbon-inducible enzyme activity and is not seen in the liver of mice which are genetically nonresponsive to aromatic hydrocarbons. (17) This genetic response to polycyclic hydrocarbons can be detected transplacentally in utero and in fetal cell culture. (18) Benz[a]anthracene and α-naphthoflavone are competitive inhibitors of metabolism of the carcinogen 7,12-dimethylbenz[a]anthracene. (19) The metabolite 3-hydroxybenzo[a]pyrene, as is the case with the parent compound benzo[a]pyrene, transforms fetal hamster fibroblasts. (20) In hamster cell cultures aneuploidy is associated with transformation, whereas chromatid breaks are correlated with polycyclic hydrocarbon-produced toxicity. (21) Mouse skin tumorigenesis initiated by 7,12-dimethylbenz[a]anthracene or benzo[a]pyrene and promoted by repeated applications of phorbol ester is unrelated to genetic differences in the extent of aryl hydrocarbon hydroxylase induction by aromatic hydrocarbons in C57BL/6N, DBA/2N, NZW/BLN inbred or hybrid mice. (22) In somatic fused-cell hybrids formed between mouse 3T3 fibroblasts in which the hydroxylase is inducible and rat HTC cells in which no basal or inducible hydroxylase is detectable, the enzyme induction by aromatic hydrocarbons ranges from the same as, to more than 20-fold greater than, that in the 3T3 parent. (23) Induction of hydroxylase activity by 3-methylcholanthrene in genetically responsive mice is associated with a conversion of low-spin to high-spin P_{450}, as measured by n-octylamine binding. (24) Polycyclic hydrocarbons, flavones, and 2-phenylbenzothiazoles are included in the class of compounds evoking hydroxylase induction in genetically responsive animals. (25) In vitro, various compounds such as flavones, 2,5-diphenyloxazone, and Lindane inhibit the polycyclic hydrocarbon-inducible hydroxylase activity which reflects high-spin P_{450} iron in the active site and affect only slightly the control enzyme activity, whereas other compounds including phenylimidazoles, SKF-525A, metyrapone, p,p'-DDT, 17-β-estradiol, delta-9-tetrahydrocannabinol, pyridine, and n-octylamine preferentially inhibit the control and phenobarbital-induced hydroxylase system and the enzyme from 3-methylcholanthrene-treated genetically nonresponsive mice (i.e., low-spin P_{450} iron), and affect only slightly the 3-methylcholanthrene-inducible enzyme. (26) In fetal mouse cell cultures treated with highly purified homologous interferon, a relationship exists between antiviral activity produced and a stimulation in the rate of hydroxylase induction by polycyclic hydrocarbons or phenobarbital.

Significance to Biomedical Research and the Program of the Institute: Phenobarbital is the most commonly used drug from a class of more than 200 drugs,

chemicals, and insecticides that induce microsomal enzymes apparently by the same mechanism. Administration of any of these substances to the pregnant animal causes transplacental induction of these mixed-function oxygenases in the fetal liver. These enzyme systems metabolize steroids, hemin, cholesterol, fatty acids, indoles, sympathomimetic amines, thyroxine, and bilirubin, as well as lipophilic foreign substrates. Administration of polycyclic hydrocarbons to the pregnant animal produces induction of this enzyme activity in the placenta and also transplacental stimulation of the hydroxylase in several fetal tissues. Cigarette smoking is associated with increases in human placental aryl hydrocarbon hydroxylase activity. Consequently, studies involving the development and extent of induction of mixed-function oxygenases in fetal tissues and in the placenta during various periods of gestation, are relevant to the program of this Institute in the following areas: (1) possible effects of environmental pollutants (i.e., polycyclic hydrocarbons and insecticides) and various drugs on normal fetal growth and development and on the health of the pregnant woman, (2) mechanisms of "pharmacological immaturity" of the premature and newborn, (3) mechanisms of disease of the fetus and newborn concerning oxygenative and conjugative metabolism of normal body substrates such as steroids and bilirubin, and (4) possible therapeutic and diagnostic procedures for aiding and predicting pharmacogenetic defects in the fetus and neonate.

Proposed Course of Project: (1) Further delineation of differences between mixed-function oxygenase induction by phenobarbital and that by polycyclic hydrocarbons, (2) characterization of specific and nonspecific binding of these various inducers to subcellular sites, (3) examination of the effects of hormones and nutrition on hydroxylase induction by either type of inducers, (4) study of various inhibitors on the induction process in cell culture, (5) characterization of the various components of the membrane-bound "hydroxylase" system, (6) analysis of differences in product formation and biophysical properties between the control, phenobarbital, and polycyclic hydrocarbon-induced oxygenases, and (7) extrapolation of these studies to formulate a general theory for explaining the mechanisms by which gene expression is controlled in mammalian cells.

Honors and Awards: None

Publications:

1. Nebert, D. W., and Bausserman, L. L.: Aryl hydrocarbon hydroxylase induction in cell cultures as a function of gene expression. Ann. N. Y. Acad. Sci. 179: 561-579, 1971.

2. Friedman, R. M., and Nebert, D. W.: Viral interference: The mechanism of action of interferon. In Mihich, E. (Ed.): Regulation of Cell Metabolism: Organizational and Pharmacological Aspects on the Molecular Level. New York, Academic Press, 1971, pp. 309-329.

3. Gielen, J. E., and Nebert, D. W.: Aryl hydrocarbon hydroxylase induction in mammalian liver cell culture. I. Stimulation of enzyme activity in nonhepatic cells and in hepatic cells by phenobarbital

polycyclic hydrocarbons, and 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane. J. Biol. Chem. 246: 5189-5198, 1971.

4. Nebert, D. W., and Gielen, J. E.: Aryl hydrocarbon hydroxylase induction in mammalian liver cell culture. II. Effects of actinomycin D and cycloheximide on induction processes by phenobarbital or polycyclic hydrocarbons. J. Biol. Chem. 246: 5199-5206, 1971.

5. Gielen, J. E., Goujon, F. M., and Nebert, D. W.: Genetic regulation of aryl hydrocarbon hydroxylase induction. II. Simple Mendelian expression in mouse tissues in vivo. J. Biol. Chem. 247: 1125-1137, 1972.

6. Nebert, D. W., Glujon, F. M., and Gielen, J. E.: Aryl hydrocarbon hydroxylase induction by polycyclic hydrocarbons: simple autosomal dominant trait in the mouse. Nature New Biol. 236: 107-110, 1972.

7. Benedict, W. F., Gielen, J. E., and Nebert, D. W.: Polycyclic hydrocarbon-produced toxicity, transformation, and chromosomal aberrations as a function of aryl hydrocarbon hydroxylase activity in cell cultures. Int. J. Cancer 9: 435-451, 1972.

8. Nebert, D. W., Benedict, W. F., Gielen, J. E., Oesch, F., and Daly, J. W.: Aryl hydrocarbon hydroxylase, epoxide hydrase, and 7,12-dimethylbenz[a]anthracene-produced skin tumorigenesis in the mouse. Molec. Pharmacol., in press.

9. Benedict, W. F., Nebert, D. W., and Thompson, E. B.: Expression of aryl hydrocarbon hydroxylase induction and suppression of tyrosine aminotransferase induction in somatic-cell hybrids. Proc. Nat. Acad. Sci., U.S.A., in press.

10. Nebert, D. W., and Gielen, J. E.: Genetic regulation of aryl hydrocarbon hydroxylase induction. Fed. Proc., in press.

11. Benedict, W. F.: Early changes in chromosomal structure and number during treatment of hamster cultures with transforming doses of polycyclic hydrocarbons. J. Nat. Cancer Inst., in press.

12. Nebert, D. W., Goujon, F. M., and Gielen, J. E.: Genetic regulation of mono-oxygenase activity. Proceedings of the 13th Annual Meeting of the East German Pharmacology Society, in press.

13. Nebert, D. W., Goujon, F. M., and Gielen, J. E.: Genetic expression of mono-oxygenase activity in the mouse. In: Pharmacology and Biochemical Toxicology, (Fonds de la Recherche Scientifigue Medicale: Brussells, Belgium), in press.

14. Nebert, D. W., Gielen, J. E., and Goujon, F. M.: Genetic expression of aryl hydrocarbon hydroxylase induction. III. Changes in n-octylamine binding to cytochrome P_{450}. Molec. Pharmacol, in press.

15. Goujon, F. M., Nebert, D. W., and Gielen, J. E.: Genetic expression of aryl hydrocarbon hydroxylase induction. IV. Interaction of various compounds with different forms of P_{450} and the effect on benzo[a]pyrene metabolism *in vitro*. *Molec. Pharmacol.*, in press.

Serial No. HD-LB3

1. Laboratory of Biomedical Sciences
2. Section on Molecular Structure
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Analytical Techniques and Instrumentation
a. Amino Acid Analysis
b. Automatic Monitoring of Chromatographic Experiments

Previous Serial Number: Same

Principal Investigator: E. Gross

Other Investigators: M. Morishita
J. Brown

Cooperating Units: Systems Maintenance Section DRS-BEI, NIH
Division of Computer Research and Technology, NIH

Man Years

Total:	10/12
Professional:	10/12
Others:	0

Project Description:

Objectives: (1) Determination of the amino acid composition of peptides and proteins; (2) improvement of existing amino acid analyzers and automation of techniques otherwise requiring many time-consuming operations; (3) adaptation of conventional amino acid analyzers to the separation and quantitation of unusual amino acids of microbial origin; (4) design, development, and operation of automated multiple sample applicators, the final instrument to offer adequate capacity for the continuous operation for prolonged periods of time without attendance.

Methods Employed: Ionexchange chromatography on custom resins of spherical shape, colorimetry, and electronic data processing equipment.

Major Findings: The one-column system for ionexchange chromatography of amino acids, which was a prerequisite for the rational implementation of automatic sample application, has been further refined in its application to the separation of unusual amino acid mixtures. Examples are the hydrolysates of nisin, subtilin, cinnamycin and duramycin. In all of these peptides the necessity arose to separate lanthionine, β-methyllanthionine, proline, and glutamine. For cinnamycin and duramycin the presence of the isomers of β-hydroxyaspartic acid and lysinoalanine has been firmly established. The operation of the automatic sample applicators assures optimal utilization of

amino acid analyzers. The first applicator is now in daily use for 24 hours. A second applicator, incorporating a number of modifications, has been installed and is now in test stages.

Significance to Biomedical Research and the Program of the Institute: The structural elucidation of peptides and proteins requires large numbers of amino acid analyses. The extensive use of the amino acid analyzers with automatic sample application has considerably facilitated the structural elucidation of nisin, subtilin, cinnamycin, and duramycin, all peptides with unusual amino acids. Unique degradation products of these peptides are also quickly determined by related approaches. Nisin and fragments of nisin are capable of inducing fetal resorptions in rats. The precise knowledge of the mode of action of these peptides will only be possible after characterization of the active peptides with the help of numerous amino acid analyses.

Proposed Course of Project: (1) The refinement of the design features of the applicators will be continued. Amino acid analyzers equipped with automated sample applicators will be linked to small independent computers capable of feedback and process control in order to demonstrate advanced techniques of laboratory automation and their application to protein chemistry. (2) The hydrolyses of peptides and proteins will be automated, and samples directly advanced to the automatic sample applicator for column loading. (3) Other laboratory processes will be further automated in collaboration with the Division of Computer and Research Technology. (4) The sequence analysis of peptides and proteins will be automated (a) by utilizing solid supports and (b) employing mass spectroscopic techniques.

Honors and Awards: None

Publications: None

Serial No. HD-LB14
1. Laboratory of Biomedical Sciences
2. Section on Molecular Structure
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Cell Organelles: Formation, Isolation, Characterization, and Function Under Normal and Abnormal Conditions

Previous Serial Number: Same

Principal Investigator: E. Gross

Other Investigators: H. Rehner
L. Corash

Cooperating Units: Behzad Mohit, Clinical Center, Clinical Pathology Department, NIH

Man Years

Total:	26/12
Professional:	26/12
Others:	0

Project Description:

Objectives: (1) Isolation and characterization of cell organelles, primarily of lysosomes; (2) study of the membrane of lysosomes: chemical composition, reactivity, permeability, structural enzymes; (3) constituents of lysosomes: isolation and characterization of enzymes, basic proteins, and other components; (4) correlation of lysosomal enzymes and their function with the life cycle of the cell under normal and pathological conditions; (5) analysis of lysosomal enzyme levels in the human placenta at various stages of gestation and the role they may play in human fetal development.

Methods Employed: Ultracentrifugation, zonal centrifugation, carrier-free electrophoresis, techniques of enzyme analysis, fluorescence and electron microscopy, separation techniques for biopolymers.

Major Findings: The isolation of detergent-free lysosomes from rat liver has been improved by changes in the ultracentrifugation techniques employed. Structural proteins from lysosomal membrane fractions may be purified by a combination of partition chromatography and countercurrent distribution. The lysosomal membrane contains proteins of relatively low molecular weight, possibly less than 10,000. The effect of peptides with α,β-unsaturated amino acids extends to lysosomes, causing lysis also of this membrane-limited entity. Morphological data indicate detergent-free lysosome fractions with few or no contaminating plasma membrane fragments. Lysosomes from human term placenta may be isolated by rate-dependent centrifugation.

Significance to Biomedical Research and the Program of the Institute: Lysosomes are of central significance throughout the life cycle of the cell. The large number of hydrolases present in the organelle perform numerous important functions under normal and abnormal cellular conditions. Profound physiological events, such as those of reproduction, may depend upon the availability of lysosomal enzymes. Extensive investigations of lysosomes in cells from different organs of the maternal as well as the fetal organism will contribute to the better understanding of the role of this organelle during development.

Proposed Course of Project: (1) Improvement and application of techniques for the isolation of lysosomes from different types of developing tissue; (2) isolation and characterization of lysosomal enzymes and other protein constituents from the organelle; (3) elucidation of the structure and function of the lysosomal membrane; (4) study of lysosomes under normal and abnormal conditions; (5) investigation of the effect of low molecular weight peptides and proteins on the lysosomal membrane; (6) correlation of the structure of membrane-active compounds with structural features of the lysosomal membrane; (7) investigation of the enzyme content of human placental lysosomes at various stages of gestation.

Honors and Awards: None

Publications: None

Serial No. HD-LB15

1. Laboratory of Biomedical Sciences
2. Section on Molecular Structure
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Fetal Resorption

Previous Serial Number: Same

Principal Investigator: E. Gross

Other Investigators: None

Cooperating Units: K. C. Snell, Laboratory of Pathology, NCI

Man Years

Total:	3/12
Professional:	3/12
Others:	0

Project Description:

Objectives: (1) Isolation, purification, and structural elucidation of substances capable of inducing fetal resorption; (2) correlation of the structure and mode of action of these compounds; (3) identification of the cellular constituents affected by and/or contributing to fetal resorption.

Methods Employed: (1) Microbial culturing techniques to provide compounds with effects on the feto-placental unit, (2) chromatographic and counter-current distribution techniques in purification steps, (3) enzymatic and nonenzymatic methods in combination with amino acid analysis to determine the structure of peptides affecting fetal development, (4) animal experiments to test the activity of substances for which an effect on fetal development has been predicted.

Major Findings: Nisin and H_2N-terminal fragments of nisin induce fetal resorption in rats when administered intravenously. Via the same route, these two compounds are also effective in preventing implantation by an as yet unknown mechanism. The COOH-terminal fragment of nisin is not effective. Its enzymatic degradation and inactivation is a likely event following intravenous injection. This is apparently also the case for nisin itself when it is administered orally. The gramicidins A, B, and C are also capable of inducing fetal resorption and preventing implantation.

Significance to Biomedical Research and the Program of the Institute: The regulatory mechanisms operative at many stages of intrauterine development are not fully understood. Studies with substances of novel types of structures

will serve to (1) identify physiological processes that contribute to the normal course of pregnancy, (2) detect undesirable metabolites which affect gestation adversely, and (3) offer chemotherapeutic approaches to pregnancy regulation.

Proposed Course of Project: (1) Structural elucidation of peptides which affect fetal development; (2) correlation of structure and function during this physiological event; (3) synthesis of peptides in which the structural elements of nisin and the physiological properties of the gramicidins are combined; (4) investigation of dose levels, routes of administration, and timing of administration of substances that affect fetal development; (5) study of the effect of previously encountered resorptions on subsequent pregnancies; (6) extension of these studies to primates.

Honors and Awards: None

Publications: None

Serial No. HD-LB5

1. Laboratory of Biomedical Sciences
2. Section on Molecular Structure
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Synthesis of Biologically Active Peptides

Previous Serial Number: None

Principal Investigator: E. Gross

Other Investigators: K. Noda

Cooperating Units: M. B. Lipsett, Reproduction Research Branch, NICHD
E. Blout, Harvard Medical School, Boston, Massachusetts

Man Years

Total:	15/12
Professional:	15/12
Others:	0

Project Description:

Objectives: (1) Synthesis of peptides with biological activity, such as (a) effects on fetal development and (b) hormone releasing properties; (2) testing and evaluating the biological properties of synthetic peptides.

Methods Employed: (1) Conventional techniques of peptide synthesis, (2) solid phase methods of peptide synthesis, (3) chromatographic and countercurrent distribution techniques for separation and purification, (4) animal experiments and bio-assays in the biological evaluation of the synthetic products.

Major Findings: Gramicidin B synthesis in a commercial peptide synthesizer in less than 60 hours, gramicidin C synthesis by solid phase techniques in a synthesizer of own design, gramicidin C synthesis with amino acid exchange: p-iodophenylalanine in place of phenylalanine. This derivative is to be crystallized for x-ray analytical studies. Synthesis of gonadotrophin-releasing factor and of partial and modified structures of this decapeptide.

The gramicidins B and C showed the full complement of biological activity on membranes. They were also fully active in their antibiotic action against a number of microorganisms.

Significance to Biomedical Research and the Program of the Institute: The highly hydrophobic gramicidins A, B, and C constitute a unique group of peptides. Their alternating pattern of L and D-amino acids allows the formation of specific types of helices which, in turn, are capable of acting

as ion-transporting transmembrane channels. The gramicidins A, B, and C affect fetal development in the rat. They also interfere with implantation. The hormone-releasing factors are of great interest as potential fertility regulating agents.

Proposed Course of Project: (1) Synthesis of analogues of gramicidin A, B, and C; (2) synthesis of radio-labeled gramicidins; (3) synthesis of nisin, fragments of nisin, and analogs of these peptides with and without radioactive amino acids; (4) synthesis of hormone-releasing factors of peptide structure.

Honors and Awards: None

Publications: None

Serial No. HD-LB19

1. Laboratory of Biomedical Sciences
2. Section on Molecular Structure
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Chemical Modification of Amino Acids. Selective Nonenzymatic Cleavage of Peptide Bonds. Structure-Function Relationship of Biologically Active Peptides and Enzymes.

Previous Serial Number: Same

Principal Investigator: E. Gross

Other Investigators: J. L. Morell
M. Morishita
J. Brown

Cooperating Units: L. C. Craig, Rockefeller University, New York, New York
F. H. White, Jr., Laboratory of Biochemistry, NHLI
P. Kohler, Reproduction Research Branch, NICHD
E. D. Becker, Laboratory of Physical Biology, NIAMD
E. Scoffone, University of Padova, Padova, Italy

Man Years

Total:	33/12
Professional:	33/12
Others:	0

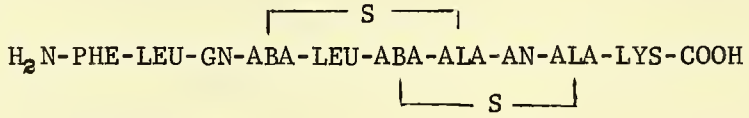
Project Description:

Objectives: (1) Chemical modification of reactive groups in polyfunctional amino acids; (2) selective cleavage of peptide bonds (nonenzymatic fragmentation) by application of chemical reactions; (3) production, isolation, and purification of biologically active peptides and enzymes; (4) fragmentation of biologically active peptides and enzymes with the objective of studying residual activity; (5) synthesis of enzyme inhibitors and study of inhibitory effects, identification of the site of inhibition and of loci responsible for enzyme activity; (6) structural elucidation of biologically active peptides and correlation of structure and function.

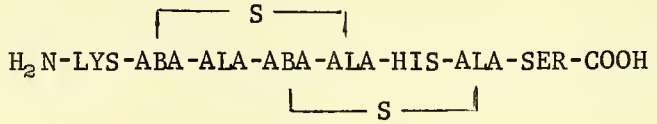
Methods Employed: (1) Enzymes; (2) chemical reagents for nonenzymatic fragmentation; (3) high energy radiation techniques; (4) photochemical techniques; (5) advanced separation and analytical techniques: chromatography, electrophoresis, countercurrent and counter double current distribution, automatic sample application in amino acid analysis, electronic equipment in data acquisition and processing; (6) nuclear magnetic resonance; (7) culturing techniques and bioassays.

Major Findings: (1) Nisin: The structure has been established as that of a pentacyclic heterodetic peptide (Figure 1). (2) Subtilin: Subtilin has in common with nisin the presence of two residues of dehydroalanine and one residue of β-methyldehydroalanine. The heterodetic bicyclic (partial) structure of thirteen ring members only, earlier seen in nisin, occurs also in subtilin:

In subtilin:



In nisin:



The partial structure of subtilin, at this stage of the investigation, is shown in Figure 2. (3) Exposure of the COOH-terminal fragment of nisin to high-energy radiation in the presence of tritium results in the incorporation of this label into serine and histidine, a fact remotely indicative of a possible catalytic role of nisin. (4) Nisin undergoes photochemical changes which may be attributed to fragmentation at the double bonds of the α,β-unsaturated amino acids. (5) Nisin affects the synthesis of human corionic gonadotrophin in trophoblast culture. (6) Large quantities of nisin and fragments of nisin have been purified by countercurrent and counter double current distribution for studies of their effects on fetal growth. (7) The peptide antibiotics cinnamycin and duramycin contain lanthionine and β-methyllanthionine and were suspects for the presence of α,β-unsaturated amino acids. The two peptides are free of α,β-unsaturated amino acids. However, they contain addition products of amino acids across the double bond of dehydroalanine and/or β-methyldehydroalanine. (8) The cyanogen bromide reaction has been applied successfully to the fragmentation of pepsinogen. In a two-step approach, methionylpeptide bonds were cleaved first. After reduction of the disulfide bonds and alkylation of the sulfhydryl groups, cyanogen bromide converted one of two S-methylcysteine residues selectively to serine and brought about the N → O-acyl shift. The earlier difficulty with the O → N-acyl shift has been overcome by the application of readily removable acyl groups in the acylation of the liberated amino group.

Significance to Biomedical Research and the Program of the Institute: The detailed knowledge of the structure of nisin and of other peptides with α,β-unsaturated amino acids is of great importance in view of the biological properties of these biopolymers. The implied working hypothesis of the interaction of α,β-unsaturated amino acids with vital sulfhydryl groups may well be of broad biological significance. On the other hand, this reaction is reversible and linked to the formation of ketoacids and amides, frequently encountered constituents of many biological systems. These structural considerations form the basis for the study of the effect of nisin and related peptides on fetal development.

Proposed Course of Project: (1) Continued exploration and application of nonenzymatic methods to the structural elucidation of peptides and proteins: (a) application of selective oxidants to the cleavage of tryptophyl peptide bonds; (b) study of the selective suppression of the cleavage of methionyl peptide bonds upon treatment with cyanogen bromide; (c) fragmentation of reduced and S-alkylated peptides and proteins; fragmentation of peptides and proteins subsequent to mercaptan addition to α,β-unsaturated amino acids; direct fragmentation at α,β-unsaturated amino acids, at time preceded by β-elimination; (d) the fragmentation at aspartic acid residues after esterification and reduction to be developed to a generally applicable method of nonenzymatic fragmentation. (2) Nisin: Continued structural elucidation of nisin-like peptides from Streptococcus lactis. (3) Subtilin: Elucidation of the primary structure; study of phylogenetic aspects of the structure of peptides containing lanthionines and α,β-unsaturated amino acids. (4) Cinnamycin and Duramycin: Structural elucidation and structural correlation. (5) Preparation of peptides with activities on fetal growth.

Honors and Awards: None

Publications:

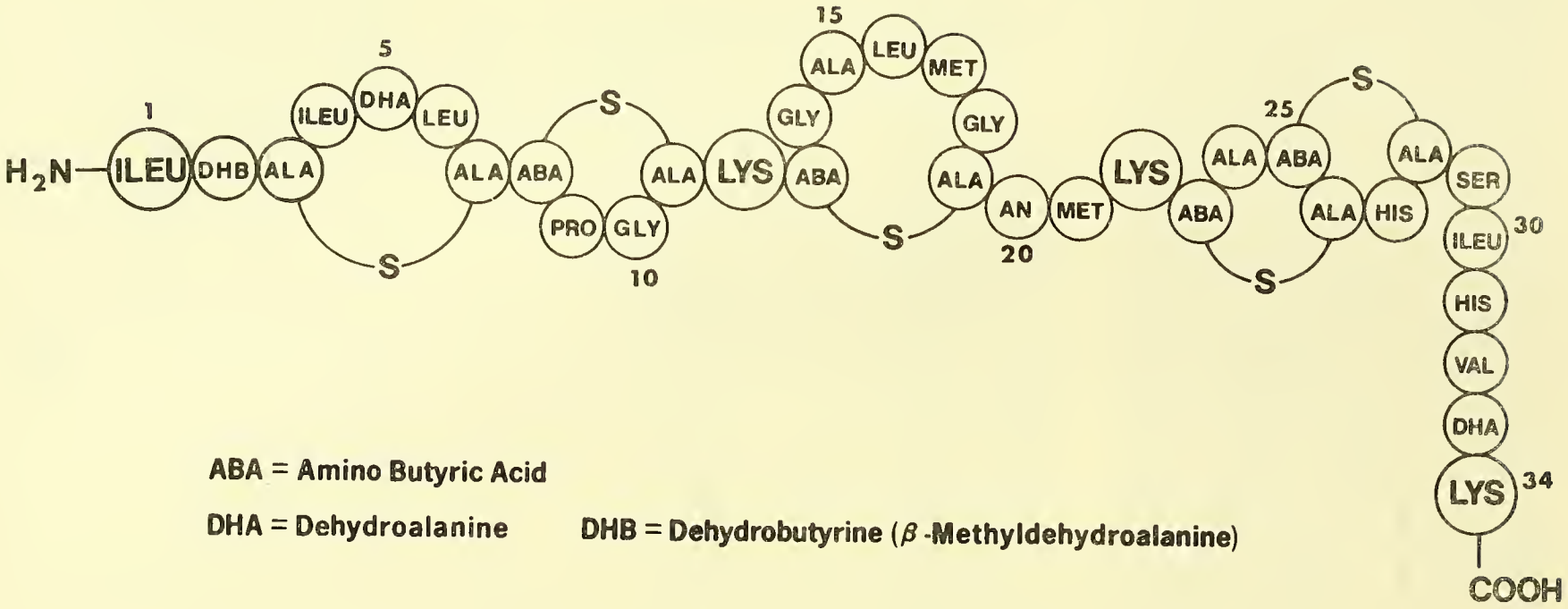
1. Gross, E., and Morell, J. L.: Peptides with α,β-unsaturated amino acids. In Scoffone, E. (Ed.): Proceedings Tenth European Peptide Symposium, Abano Terme, Italy, September 7-13, 1969. North-Holland Publishing Company, 1971, pp. 356-360.

2. Gross, E., and Morell, J. L.: The structure of nisin. J. Amer. Chem. Soc. 93: 4634-4635, 1971.

3. Gross, E.: Structure and function of peptides with α,β-unsaturated amino acids. Intra-Science Chemical Reports 5: 405-408, 1971.

4. Gross, E., and Kiltz, H.-H.: The number and nature of α,β-unsaturated amino acids in subtilin. Biochem. Biophys. Res. Commun., in press.

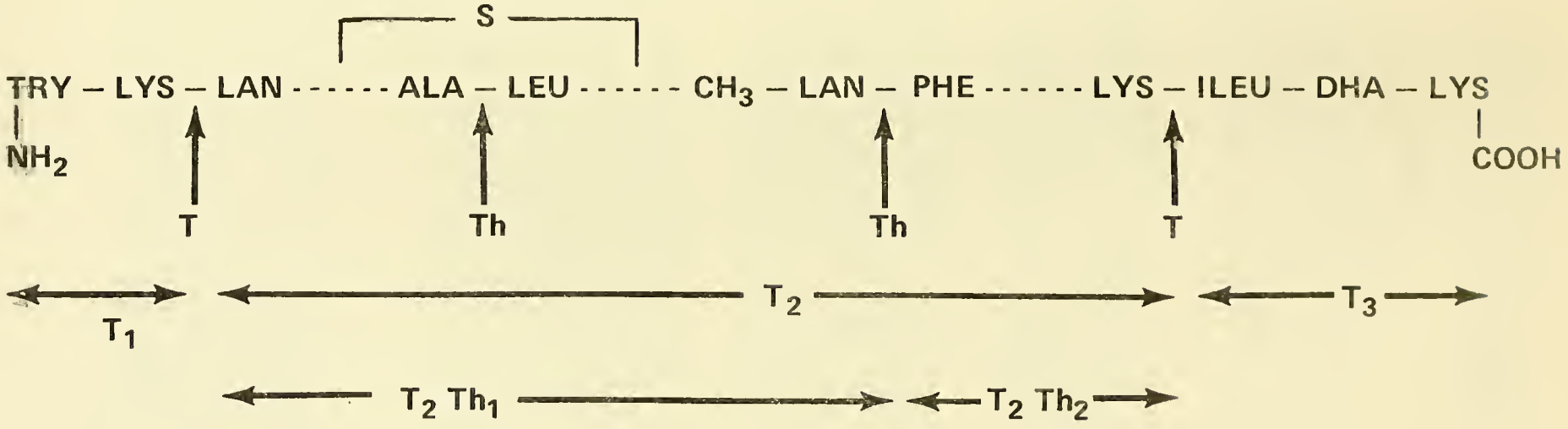
THE STRUCTURE OF NISIN



ABA = Amino Butyric Acid

DHA = Dehydroalanine DHB = Dehydrobutyrine (β-Methyldehydroalanine)

PARTIAL STRUCTURE OF SUBTILIN



T = TRYPSIN Th = THERMOLYSIN

NICHD Annual Report
July 1, 1971 through June 30, 1972
Pregnancy Research Branch

SUMMARY

The Pregnancy Research Branch became operational this fiscal year with a staff comprised of three professional and four nonprofessional persons. Most of the initial activity of the branch was directed at the equipping and remodeling of laboratories in the temporary off campus site. Now that this is completed, research has begun and is progressing well in several areas.

The research protocols reflect a continuation of studies already in progress before the branch was established as well as several new projects. The majority of experiments require the use of pregnant animal models. Via purchase contract mechanisms, we were successful in obtaining a sufficient number of timed gestations in baboons and sheep as well as a few pregnant Rhesus monkeys. This critical resource for our research has also been secured for the coming fiscal year.

The use of the pregnant Rhesus monkey for examination of normal maternal-fetal-placental carbohydrate metabolism in the third trimester as well as the adverse effects of induced maternal glucose intolerance has continued. Oral glycemic stimuli to the intrauterine near term fetus have not been associated with fetal pancreatic beta cell responsiveness nor changes in baseline fetal plasma glucagon levels. This finding, both in normal and abnormal pregnancy, is in contrast to the change in pancreatic responsiveness found when intravenous glycemic stimuli are applied directly to the fetus.

The determination of factors regulating placental steroid synthesis in pregnancy has continued in both the human and baboon with some important findings. The importance of precursor availability on maternal urinary estrogen concentrations has been demonstrated by the intra-amniotic administration of dehydroepiandrosterone. The ability of endogenous steroid levels to reduce effective levels of placental steroid sulfatase and thus regulate estrogen synthesis is now confirmed. The appropriateness of using the baboon as a model for estrogen synthesis in human pregnancy is supported by the findings of preferential conversion of nonconjugated derivatives, the fact that estradiol-17β plays a central role, and the susceptibility of sulfatase to inhibition.

New protocols have begun in several areas. The use of acute and chronic fetal-maternal catheter preparations in sheep pregnancies has permitted an examination of the effects of general and conduction anesthesia as well as induced changes in blood pH on fetal blood flow and fetal oxygen xonsumption. Sequential samples of amniotic fluid in both baboon and monkey pregnancies reveal a similar progression of phospholipid/syphingomyelin changes near term to those found in human pregnancies. In an attempt to identify the source of these two fats in amniotic fluid,

ligation of the fetal trachea and/or esophagus has been performed in both the baboon and the monkey during the second trimester. Amniotic fluid samples from these pregnancies are now being analyzed. A new method to destroy the fetal pituitary in utero without interrupting the pregnancy has been devised. This method relies on radiofrequency lesions delivered through a probe inserted into the sella turcica via the orbit. The effects of fetal hypophysectomy on length of gestation and adrenal-gonadal histology will be examined.

The etiology of the tenfold concentration of prolactin in human amniotic fluid compared to maternal and fetal plasma concentrations has been examined by the use of radioisotopically labelled human prolactin three-compartmental transfer in monkey pregnancies. The placenta is impermeable to and does not transfer prolactin from fetus to mother or mother to fetus. Furthermore, the prolactin present in amniotic fluid transfers to either the mother or the fetus at an extremely slow rate. Finally, radioimmunoassay methods have been developed for the measurement of parathormone and thyrocalcitonin concentrations in monkey plasma. Accordingly, several aspects of calcium metabolism in pregnancy have been approached by the examination of the bidirectional placental transfer of these two hormones as well as the effects of plasma calcium perturbations on the levels of these two hormones. A major thrust in this area is planned this year. In addition, a search for a satisfactory chronic preparation in the subhuman primate will be started. This is a critical technical step if these animals are to be used for interpretation of human clinical events with more optimal efficiency.

Clinically, the branch has become involved in the medical activities of George Washington University Medical School, Georgetown Medical School, and D.C. General Hospital. This contact with indigent patients, obstetrical residents, and medical students will provide a more knowledgable basis for eventual exchange and collaboration when this branch's clinical activities begin on the NIH reservation. In addition, several meetings have been held with the Maternal and Child Health Division of the Montgomery County Health Department and a role is anticipated in the care of their prenatal patients.

This branch is anticipating a change of quarters to the NIH reservation. It is felt that the resultant ease of access and ease of communication will enhance all aspects of our activities. A new animal surgical suite as well as patient facilities are now well into the planning stage and construction is anticipated soon. Success in professional recruitment and enlargement of the staff will permit a fuller realization of our potential contributions to pregnancy research.

Serial No. HD-PR1
1. Pregnancy Research Branch
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Carbohydrate Metabolism during Primate Pregnancy

Principal Investigator: Ronald A. Chez, M.D.

Other Investigators: Daniel H. Mintz, M.D., University of Miami; Antoinette Schenk, University of Miami; Donald L. Hutchinson, M.D., University of Pittsburgh; I. Arthur Mirsky, M.D., University of Pittsburgh; Hernando Salazar, M.D., University of Pittsburgh; Rosemary Fox, University of Pittsburgh; Patrick Moran.

Cooperating Units: Experimental Surgery & Clinical Medicine Section, Laboratory Aids Branch, Division of Research Services.

Man Years:

Total:	2.9
Professional:	1.0
Other:	1.9

Project Description:

Objectives:

1. To exam the effect or oral glycemic stimuli on fetal pancreatic beta cell responsiveness.
2. To determine if glucagon is transferred across the primate placenta and if hyperglucagonemia exists in pregnancies associated with maternal carbohydrate intolerance.
3. To compare a monkey pregnant model of induced diabetes mellitus with data presently available from human pregnancies associated with diabetes mellitus.

Methods Employed: Accurately timed gestations in Rhesus monkeys are used. Streptozotocin, 40-48 mg/kg, is administered intravenously to the adult female prior to or in the first trimester of pregnancy. This drug induces a glucose intolerant state secondary to pancreatic beta cell destruction. During the third trimester, acute experiments are performed under general anesthesia and aseptic surgical techniques. Cannulae are placed into both the maternal and fetal circulations to permit sequential sampling of blood. A catheter is placed transuterine into the fetal stomach via the mouth and glucose solution is injected into the stomach lumen. Blood aliquots are analyzed for glucose, free fatty acids, insulin growth hormone, and glucagon

using biochemical and radioimmunoassay procedures. After performing the same experiment on the newborn in the first day of life, tissue is examined histologically from both mother and conceptus.

Major Findings: The primary focus of this work has been in progress for several years. The results to date are summarized in four articles. J. Clin. Invest. 48:176, '69, J. Clin. Invest. 49:1517, '70, J. Clin. Invest. Metab. 20:805, '71, J. Clin. Invest. 51:837, '72. The work done this year has so far demonstrated a failure of orally administered glucose to enhance plasma insulin concentrations in either the normal or abnormal fetus and a lack of hyperglucagonemia during fasting or induced hyperglycemia in the normal or abnormal. Histologically, fetal beta cell hyperplasia has been found in the diabetic model.

Significance to Bio-Medical Research and to the Institute: Prior to this study, the interrelationships of fetal and maternal glucose, insulin, growth hormone, and glucagon could only be conjectured in primate pregnancy. The success of the streptozotocin diabetic model has validated the theory of fetal hyperinsulinism in diabetic pregnancies. In question is the singular significance of maternal euglycemia in the measurement of diabetic pregnancies if maternal hyperaminoacidemia coexists. The therapeutic implications for human pregnancies are primarily in the area of basic mechanisms for the early induction of fetal beta cell responsiveness to glycemic and amino acid stimuli prior to term delivery.

Proposed Course: The absence of a chronic fetal preparation in the monkey limits the direction of this protocol. Methods are presently being evaluated to measure the cylic AMP content of fetal beta cells and to culture and grow primate fetal beta cells in vitro. The effects of this induced carbohydrate intolerance state on adult monkey retina and muscle capillary basement membranes are now being investigated.

A research contract with John E.A. Tyson, M.D., Johns Hopkins University has now been negotiated. The objectives of this contract are:

A. To study the effects of acute starvation induced in the first half of pregnancy on human maternal plasma placental lactogen concentration.
B. To study the relationship between carbohydrate-insulin-growth hormone metabolism and human maternal plasma and amniotic fluid concentrations of prolactin.
C. To measure the concentrations of the essential and glucogenic amino acids in human maternal plasma, fetal plasma, and amniotic fluid under conditions of acute maternal starvation and/or increments in maternal plasma placental lactogen concentration.

It is anticipated that protocols for the subhuman primate will be generated by the data evolving from this work.

Honors and Awards: Ronald A. Chez

Elected to Endocrine Society, 1971.
Member, Editorial Board, Journal of Medical Primatology, 1971.
Appointed Clinical Professor, Department Ob-Gyn, George Washington University

School of Medicine, Washington, D.C., 1971.
Appointed Clinical Professor, Department Ob-Gyn, Georgetown University School of Medicine, Washington, D.C., 1971.
Appointed Obstetrical Consultant, D.C. General Hospital, Washington, D.C., 1971.
Invited Speaker, Perinatal Research Society Meeting, Charlottesville, Virginia, September, 1971.
Invited Speaker, Sixth Rochester Trophoblast Conference, Rochester, New York, October, 1971.
Invited Lecturer, George Washington Obstetrical Society, Washington, D.C., November, 1971.
Visiting Professor, Nuffield Institute for Medical Research, Oxford, England, February 1972.
Invited Lecturer, Walter Reed General Hospital, Washington, D.C., April, 1972.
Invited Lecturer, Twentieth Annual Clinical Meeting, The American College of Obstetricians and Gynecologists, Chicago, Illinois, May, 1972.
Invited Lecturer, Semi-Annual Diabetes in Pregnancy Workshop, University of Southern California Medical Center, Los Angeles, California, May, 1972.
Invited Speaker, IV International Congress of Endocrinology, June, 1972.

Publications: Chez, R.A.: Models and Midwives., J. Med. Prim. 1:73-74, 1972.

Publications not reflected on 1971 curriculum vitae:

Chez, R.A., Mintz, D.H., Hutchinson, D.L.: The Effect of Theophylline on Glucagon and Glucose Mediated Plasma Insulin Responses in the Subhuman Primate Fetus and Neonate., Metabolism 20:805, 1971.

Chez, R.A., Josimovich, J.B., Schultz, S.G.: The Transfer of HPL Across Isolated Amnion-Chorion., Gynec. Invest. (In Press).

Chez, R.A., Mintz, D., Hutchinson, D.L., Horger, E.O.III.: Third Trimester Simian Fetal Carbohydrate Metabolism., In International Symposium on Physiological Biochemistry of the Fetus, Hodari., Charles C. Thomas, Springfield, 1971. (In Press).

Chez, R.A., Mintz, D.H., Horger, E.O. III, Hutchinson, D.L.: The Placental Transfer of Polypeptide Hormones Related to Carbohydrate Metabolism., Proc. 2nd Conf. exp. Med. Surg. Primates, P578, Moor-Jankowski, Karger, Basel, 1971.

Mintz, D.H., Chez, R.A., Hutchinson, D.L.: Primate Fetal Beta Cell Responsiveness in Induced Diabetes Mellitus., J. Clin. Invest. 51:837, 1972.

Cohn, M.L., Chez, R.A., Hingson, R.A., Szulman, A.E., Trimmer, M.: Use of Jet Insulin Injection in Diabetes Mellitus Therapy., Diabetes, 21:39, 1972.

Chez, R.A.: Mental Disability as a Basis for Contraception and Sterilization., Social Biology 18:S120, 1971.

Chez, R.A.: The Role of the Pharmacist in Family Planning: A Pennsylvania Survey., J. Amer. Pharm. Assoc. (In Press).

Chez, R.A.: Student Objectives, Didactic Resources, and Faculty Models in Family Planning Education., In Macy Conference on Family Planning, Demography, and Human Sexuality in Medical Education, Lippard, 1971.

Chez, R.A.: Movies of Human Sexual Response as Learning Aids for Medical Students., J. Med. Educ., 46:977, 1971.

Chez, R.A., O'Gorman, D.J.: Evaluation of an Audiovisual Carrel-Tutorial Course in Clinical Medicine., Did. Medica, 1972 (In Press).

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Steroid Hormones in Primate Pregnancy

Principal Investigator: John D. Townsley, Ph.D.

Other Investigators: C. Deans Crystle, M.D., Elliece Smith, B.S., Linda Gartman, Norman Dubin, Ph.D., University of Maryland; George Granis, Ph.D., Ohio State University.

Cooperating Units: Experimental Surgery & Clinical Medicine Section, Laboratory Aids Branch, Division of Research Services.

Man Years:

Total:	4.2
Professional:	2.2
Other:	2.0

Project Description:

Objectives:

1. To ellucidate the mechanisms regulating steroid hormone synthesis and metabolism during human pregnancy.
2. To evaluate and use the baboon as a model for such studies.
3. To compare the utility of estrogen determinations in maternal urine and serum for the assessment of fetal placental status in the high-risk pregnancy.

Methods Employed: The project demands a variety of complementary approaches ranging from metabolic studies in the intact pregnant primate to determination of activities of steroidogenic enzymes in sub-cellular fractions of placenta. Analytical methods include fluorometric, colorimetric, and radioimmunoassays for estrogen in body fluids in double isotope techniques for kinetic enzyme assays and the metabolic studies in vivo.

Major Findings: Intra-amniotic administration of dehydroepiandrosterone sulfate to normal pregnant women results in increased maternal serum and urinary estrogen concentrations indicating that precursor availability is a primary determinant of estrogen production. Placental steroid sulfatase is the enzyme that catalyzes the initial reaction in utilization of extra placental estrogen precursors. Previously we proposed that endogenous steroids may reduce effective levels of placental steroid sulfatase and

thus regulate estrogen synthesis when excess precursor is available. Our recent studies confirm that this is so utilizing the requisite criteria of such a physiological control mechanism which are that such inhibition is reversable, readily demonstrable in physiological concentrations, and is specific. Therefore, there are clearly defined structured-activity correlations.

Estrogen synthesis during pregnancy in the baboon closely resembles that found in women in several important respects. Circulating precursors are increasingly utilized with preferential conversion of non conjugated derivatives and estradiol-17β plays the centrol role. Although little estriol is actively formed, its mode of biosynthesis appears similar to that in women. Susceptibility of sulfatase to inhibition suggests that similar placental regulating mechanisms may be present in this subhuman primate species.

Although serum determinations offer distinct practical advantages, preliminary analysis of data indicate that serum estriol assays are not inherently superior to urinary estrogen measurements in screening high-risk pregnancies. Contrary to previous reports we have shown significant diurnal changes in maternal serum and urinary estrogen levels and therefore correlate with the well documented rhythm of adrenal cortical steroid levels.

Significance to Bio-Medical Research and to the Institute: Because of the widespread utilization of steroid hormone assays throughout pregnancy and their relationship to fetal-placental well being an understanding of the mechanisms controlling their synthesis and metabolism is essential for definitive resolution of many problems of reproductive failure. Our approaches provide fundamental knowledge of these processes and practical information of immediate use to the clinician. Further elucidation of controlling mechanisms will require manipulative procedures in both the mother and the fetus which are precluded in humans. An adequate animal model must be found in order to do this and our studies indicate that the baboon appears to be a promising candidate.

Proposed Course: Using intact tissue preparations rather than the cell-free systems studied heretofore, additional placental enzymatic control mechanisms will be examined. The potential physiological significance of such regulatory factors in human placenta will be assessed in this manner. The significance of precursor availability in regulating estrogen synthesis by the pregnant baboon will be examined further and a relative importance of fetal and maternal contributions to the precursor pool assessed. These studies will require adaptation of existing estrogen radioimmunoassay procedures to baboon body fluids. Further we will have to initiate exploration of the perinatal maturation of the baboon fetal adrenal-pituitary-hypothalamic access. The comparison of serum and urinary estrogen determinations in the assessment of fetal placental status will be completed during the coming year.

Honors and Awards: John D. Townsley

Invited Lecturer, W.H.O. Symposium on the Use of Nonhuman Primates for

Research on Problems of Human Reproduction, Sukhumi, U.S.S.R., December, 1971.
Invited Lecturer, Fifth Meeting of the International Study Group for Steroid Hormones, Rome, Italy, December, 1971.
Invited Lecturer, Department of Nutrition, University of Maryland, February, 1971.
Invited Lecturer, Department of Obstetrics and Gynecology, University of Maryland, March, 1971.

Publications:

Brodie, H.J., Hay, C.E., Townsley, J.D.: Microbiological Hydroxylation of estr-4-ene-3,17-dione., Biochim. Biophys Acta 239:103, 1971.

Townsley, J.D.: The Baboon as a Model for Studies on Estrogen Synthesis., Acta Endocrinologica 1972 (In Press).

Kling, O.R., Rubin, E.J., Townsley, J.D.: Estrogen Synthesis by the Pregnant Baboon., J. Med. Prim. 1:102, 1972.

Townsley, J.D., Rubin, E.J.: Structure-Activity Correlations for Steroid Inhibition of Human Placental Steroid 3-Sulfatase., Research on Steroids. 5, 1972 (In Press).

Honors and Awards: C. Deans Crystle

Appointed Research Associate, Department Obstetrics and Gynecology, Georgetown University, Washington, D.C., 1971.
Appointed Assistant Clinical Professor, Department Obstetrics and Gynecology, George Washington University Clinic, Washington, D.C., 1972.

Publications: None

Serial No. HD-PR3
1. Pregnancy Research Branch
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Amniotic Fluid Fat Concentrations in Primate Pregnancy

Principal Investigator: Ronald A. Chez, M.D.

Other Investigators: Louis Gluck, M.D., University of California, La Jolla; Patrick Moran.

Cooperating Units: Experimental Surgery & Clinical Medicine Section, Laboratory Aids Branch, Division of Research Services.

Man Years:

Total:	0.25
Professional:	0.10
Other:	0.15

Project Description:

Objectives:

1. To determine the patterns of amniotic fluid fat composition in monkey and baboon pregnancies.
2. To examine the effect of fetal tracheal and/or esophageal ligation on amniotic fluid phospholipid and syphingomyelin ratios.

Methods Employed: Rhesus monkeys (Macaca mulatta) and baboons (Papio papio) with accurately timed gestations are used. Transabdominal percutaneous amniocentesis is sequentially performed during pregnancy. Late in the second trimester, fetal tracheal and/or esophageal ligation is performed under aseptic surgical conditions. The fetus is delivered electively at term via cesarean section.

Major Findings: The pattern of amniotic fluid fat concentrations changes near term in the subhuman primate in a manner similar to that found in the human. The phospholipid/syphingomyelin (P/S) reverses at approximately 90% gestational age. Tracheal ligation is associated with an increase in the methylation pathway of lecithin synthesis, nondistended lungs (in contrast to the sheep), and relative oligohydramnios. For reasons that remain unclear, esophageal ligation is associated with polyhydramnios and early fetal demise.

Significance to Bio-Medical Research and to the Institute: The P/S in human amniotic fluid is a reliable diagnostic tool for the determination of fetal

lung maturity. The effects of congenital anomalies, intrauterine stress, and maternal or fetal medication remain unknown. The use of this animal model will permit research into the physiology and pathophysiology of these pathways.

Proposed Course: An attempt to induce fetal lung maturation prior to its normal occurrence will be made. There is a suggestion that intrauterine chronic asphyxia and fetal adrenal cortical activity are coregulators of fetal lung maturation. The effects of the administration of corticosteroids to the fetus and fetal adrenal-hypophysectomy will be examined.

Honors and Awards: None

Publications: None

Serial No. HD-PR4
1. Pregnancy Research Branch
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Effects of Induced Fetal Blood pH changes on Fetal Blood Flow

Principal Investigator: Ronald A. Chez, M.D.

Other Investigators: Andre Hellegers, M.D., Georgetown University; Robert Cefalo, M.D., Georgetown University; Joan Simkovich, Ph.D., Georgetown University; Patrick Moran

Cooperating Units: Division of Research Services, Veterinary Resources Branch, Animal Center Section, Ungulate Unit, NIH

Man Years:

Total:	2.6
Professional:	2.2
Other:	0.4

Project Description:

Objectives:

1. To study the effects of induced fetal metabolic acidosis on fetal blood flow, fetal oxygen consumption, uterine blood flow, and fetal and maternal plasma oxygen tensions.
2. To study the effects of induced fetal respiratory acidosis on the same parameters.
3. To determine whether permeability characteristics of placental oxygen transfer can be modified.

Methods Employed: Accurately timed gestations in Dorset sheep are utilized. Under anesthesia and asceptic surgical conditions, cannulae are placed into the maternal and fetal circulations. The latter is done without interrupting the integrity of the amniotic sac. The experiment is then performed under this acute set of conditions or 3-7 days subsequently after the animal has recovered and is in a nonstressed nonanesthesized state. Measurements are made with the autoanalyzer, Van Slyke apparatus, and blood gas-pH equipment.

Hemihysterectomy was performed in 16 sheep prior to conception; 12 of these conceived and are presently pregnant. Hemihysterectomy immediately prior to the acute experiments cited above have been done in six instances.

Major Findings: The bulk of the data is presently being analyzed. It has been determined that Halothane anesthesia to the mother significantly decreases fetal blood flow, and its use has been discontinued in this work and replaced with conduction anesthesia. Preliminary data suggests that fetal blood flow and fetal oxygen consumption are not changed in the presence of fetal alkalosis. The possibility that surgical limitation of implantation sites for cotyledons will result in intrauterine growth retardation and chronic fetal asphyxia will be examined when the animals are closer to term.

Significance to Bio-Medical Research and to the Institute: The utility of fetal heart rate, acid base, and gas monitoring in human pregnancy continues to be debated. In addition to a lack of data on the significance of some of the changes observed, there is a lack of information as to fetal therapeutic implications. Data from the pregnant sheep model has been successfully extrapolated to human pregnancy in several areas. It is believed that this project will provide physiological and therapeutic information on fetal acid-base control mechanisms, fetal blood flow, and fetal oxygen consumption.

Proposed Course: Obtaining specimens from the remainder of this year's sheep, chemical measurements and collation of data will occupy another six months. It is presently anticipated that the hemihysterectomy preparations will provide the primary thrust for experiments in this next year.

Honors and Awards: None

Publications: None

Serial No. HD-PR5
1. Pregnancy Research Branch
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Calcium Homeostasis in the Subhuman Pregnant Primate

Principal Investigator: Ronald A. Chez, M.D.

Other Investigators: Daniel H. Mintz, M.D., University of Miami; Antoinette Schenk, University of Miami; Patrick Moran

Cooperating Units: Experimental Surgery & Clinical Medicine Section, Laboratory Aids Branch, Division of Research Services.

Man Years:

Total:	1.5
Professional:	0.6
Other:	0.9

Project Description:

Objectives:

1. To examine the bidirectional placental transfer of radioisotopically labelled bovine parathyroid hormone and synthetic human calcitonin.
2. To study the effects of perturbations of maternal and fetal serum calcium and phosphorous on the serum calcium, ionized calcium, and immunoreactive parathyroid hormone and calcitonin in the fetal and maternal circulations.
3. To study the effects of the intravascular administration of PTH and CT on placental transport of radiolabelled calcium.
4. To investigate the hormonal control of calcium homeostasis in the neonate and the pathophysiology associated with neonatal tetany.

Methods Employed: Rhesus monkeys (Macaca mulatta and Macaca speciosa) with accurately timed gestations are used. Under aseptic surgical conditions, cannulae are placed into the fetal and maternal circulations. An attempt is made to maintain the integrity of the amniotic sac. Highly purified bovine PTH or synthetic human CT iodinated with radioactive iodine, calcium gluconate, or EGTA are intravenously administered to mother or fetus. Blood aliquots are sequentially obtained for appropriate measurements utilizing atomic absorption spectrometry, flow through electrodes, and radioimmunoassay.

Major Findings: Preliminary data suggests that a limited bidirectional placental transfer of PTH occurs in the third trimester, and that CT does

not cross the placenta. The infusion of calcium gluconate or EGTA into nonpregnant monkeys and baboons is associated with the same changes in plasma PTH as found in adult humans.

Significance to Bio-Medical Research and to the Institute: Pregnancy elicits a physiologic adaptive response in the mother to protect a late pregnancy fetal claim on calcium for mineralization of the fetal skeleton. There is a paucity of decisive data in human pregnancy secondary to the inherent experimental difficulties encountered in studying healthy patients and the lack until recently of reliable radioimmunoassay techniques for the measurement of plasma levels of parathormone and thyroid calcitonin. The utilization of the subhuman primate permits investigation of this complex problem with techniques which permit study of the fetus in utero and assay procedures that can be performed on small aliquots of plasma. It is hoped that the data generated will provide the first definitive exploration in primates of the parathyroid-calcitonin releasing mechanism studied simultaneously in both fetus and mother.

Proposed Course: All aspects of the project require exploration and a major commitment to this project is planned. The obtaining of data will depend upon the availability of pregnant monkeys now that the radioimmunoassay and ionized calcium techniques have been developed.

Honors and Awards: None

Publications: None

Serial No. HD-PR6
1. Pregnancy Research Branch
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Prolactin Metabolism During Primate Pregnancy

Principal Investigator: Ronald A. Chez, M.D.

Other Investigators: John E.A. Tyson, M.D., Johns Hopkins University; Janice Huth, Johns Hopkins University; Beverly Smith, Johns Hopkins University; Prudence Thomas, Johns Hopkins University; Patrick Moran.

Cooperating Units: Experimental Surgery & Clinical Medicine Section, Laboratory Aids Branch, Division of Research Services.

Man Years:

Total:	2.1
Professional:	0.4
Other:	1.7

Project Description:

Objectives: To study the bidirectional placental and amniotic fluid transfer of prolactin in primate pregnancy.

Methods Employed: Seven Rhesus monkeys (Macaca mulatta and Macaca speciosa) with accurately timed gestations have been used. Under aseptic surgical conditions, cannulae are placed into the maternal and fetal circulations as well as the intact amniotic sac. Highly purified human prolactin iodinated with radioactive isotopes is injected into one of the three compartments. Sequential aliquots of blood and amniotic fluid are examined for hot and cold prolactin concentrations using radioimmunoassay and chromatography techniques.

Major Findings: Prolactin does not cross the primate placenta in either direction in the third trimester. The prolactin in amniotic fluid is only partially of maternal origin and not of fetal origin. Analyses of actual transfer kinetics are still being performed.

Significance to Bio-Medical Research and to the Institute: The amniotic fluid concentration of prolactin is approximately ten times that of the levels found in maternal and fetal plasma. Prolactin has been shown to affect the transfer of sodium across the gills of fish and the renal tubules of dogs. The role of prolactin in the exchange of water and electrolytes across fetal membranes needs to be clarified. This study describes some of the fundamental

physiology necessary as a foundation.

Proposed Course: Further use of the intact primate pregnancy is not planned. Under consideration is a protocol examining the effects of prolactin on sodium transfer across fetal membranes mounted in an ussing chamber.

Honors and Awards: None

Publications: None

Serial No. HD-PR7
1. Pregnancy Research Branch
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Effects of Fetal Hypophysectomy in Primate Pregnancy

Principal Investigator: Ronald A. Chez, M.D.

Other Investigators: John Franz, M.D., NINDS; Griff T. Ross, M.D., Katherine Snell, M.D., NCI; Patrick Moran

Cooperating Units: Experimental Surgery & Clinical Medicine Section, Laboratory Aids Branch, Division of Research Services.

Man Years:

Total:	0.8
Professional:	0.6
Other:	0.2

Project Description:

Objectives:

1. To devise a technique to destroy the fetal pituitary in utero.
2. To examine the effects of fetal hypophysectomy on length of gestation.
3. To examine the lack of fetal pituitary trophic hormones on the histology of the gonads and adrenals.

Methods Employed: Accurately timed gestation in the Rhesus monkey or baboon are used. Under aseptic surgical conditions, use of specially fabricated equipment, and x-ray localization; a thin rod is placed into the fetal sella turcica via one orbit. This is done while maintaining the fetus inside the uterus. A radiofrequency lesion is then generated when the needle is in place. If spontaneous delivery does not occur, the pregnancy is allowed to go three weeks past term before cesarean section is performed. Both light and electron microscopy are used to examine the selected tissues.

Major Findings: When the monkey fetal pituitary is destroyed by ittrium 90 (Y^{90}) irradiation, prolonged gestation occurs. The fetal adrenal glands of such animals show a loss of the fetal zone and a well differentiated three layer adult cortex. There is a suggestion that primordial follicles do not develop in the ovaries. Success with the new method described above is just now being achieved and data will be forthcoming.

Significance to Bio-Medical Research and to the Institute: The factors that

determine the onset of labor at term and in the premature remain unknown. The physiologic import of fetal oxytocin to labor and fetal adrenal function to labor and maternal steroid production is not defined. Further, the need of fetal gonadotropins in the maturation of gonads prior to delivery remains uncertain.

Proposed Course: This protocol will be applied to both pregnant monkeys and pregnant baboons during the coming year. The nature of the findings and the success and mortality rate will dictate future approaches.

Honors and Awards: None

Publications: None

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Children's Diagnostic & Study Branch

This program continues to function under severe personnel restraints, with only 2 professionals. Research emphasis has been on genetic disorders and mental retardation.

Published cytogenetic studies include reports of the discovery of an infant with both the XYY chromosome anomaly and Trisomy 21 Down's Syndrome (48, XYY, G+), and of the effects of pronase on chromosomes.

Dermatoglyphics are being obtained and analyzed from a large population of Caucasians and Negroes to form an updated normal control sample for use as a comparison in other genetic studies. These other studies are evaluating the dermatoglyphics of population isolates and of individuals with genetic disorders and congenital anomalies for use as a genetic marker in the condition.

A clinical evaluation of hyperactive children on medication demonstrated marked effectiveness of dextroamphyetamine in reducing their hyperactivity, and some improvement with chlorpromazine, compared to no drug or placebo. Blood lactic acid and urinary catecholamines were not related to the child's anxiety level as assessed by observers.

Patients with Down's Syndrome have low levels of platelet serotonin. Some investigators have suggested that the mental retardation of this syndrome may be due to abnormal CNS metabolism of this neurotransmitter. In order to investigate this hypothesis, levels of platelet serotonin were assessed in a number of patients with Down's Syndrome, who then underwent lumbar puncture to assess CNS metabolism of serotonin. The studies revealed that while platelet serotonin was 61% below normal in these patients, there was no abnormality detected in CNS serotonin metabolism or transport. Both patients and controls had a 3-fold rise in CNS 5-HIAA concentration (the principal serotonin metabolite) when probenecid was administered.

Serial No. HD-CD1(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Relation Between Minor Physical Anomalies, with Dermatoglyphics and Blood Groups

Previous Serial Number: HD-CD1(c)

Principal Investigators: Chris C. Plato, M. S., Geneticist

Other Investigators: C. F. Halverson, Jr., Ph.D.
Mary F. Waldrop, M. A.
P. V. Cardon, M. D.
H. Gold, M. D.
National Institute of Mental Health

Cooperating Unit: Child Research Branch, National Institute of Mental Health, Bethesda, Maryland

Man Years

Total	:	0.01
Professional	:	0.01
Other	:	0.00

Project Description:

Objectives: This study is a search for associations between blood groups and dermatoglyphics with minor physical anomalies (such as hypertelorism, high-arched palate and durved fifth finger) and hyperactive behavior in young children.

Methods Employed: Sixty boys and girls age 7½-8½ years whose behavior was studied originally in the newborn period (1960-1962) and again during the early school period (1962-1964) were chosen for this study. These sixty children are divided into four subgroups as follows: (A) High activity associated with three or more mimor physical anomlies. (B) High activity associated with one physical anomaly. (C) Low activity associated with three or more physical anomalies. (D) Low activity associated with one physical anomaly. For blood grouping each sample was typed by commercial antisera for ABO, Rh, Kell, Duffy, and other rare systems. The dermatoglyphics were taken by the Faurot "inkless method." Both finger and palm patterns were evaluated.

Major Findings: Project still in progress.

Significance to Biomedical Research and the Program of the Institute: This pilot project is designed to search for genetic associations with hyperactive behavior and/or minor physical anomalies.

Proposed Course of Project: This pilot project has been completed. Data analysis and manuscript preparation are underway.

Honors and Awards: None

Publications: None

Serial No. HD-CD4(d)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Cytogenetic Correlates of Antisocial Behavior

Previous Serial Number: Same

Principal Investigators: W. Edwin Dodson, M.D., Children's Hospital, St. Louis, Mo.
Matti S. Al-Aish, Ph.D., Research Cytogeneticist

Other Investigators: Duane Alexander, M.D., Assistant to the Scientific Director

Cooperating Units: The Circuit Court of Prince George's County, Division of Juvenile Causes, Mr. David Gray Ross, Master of Juvenile Causes
The Juvenile Court of the District of Columbia, The Honorable Morris Miller, Chief Judge

Man Years

Total	:	0.05
Professional	:	0.05
Other	:	0.00

Project Description: Complete

Honors and Awards: None

Publications: Dodson, W.E., Al-Aish, M., and Alexander, D.: Cytogenetic survey for XYY males in two juvenile court populations, with a case report. J. Med. Genet., in press

Serial No. HD-CD8(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Dermatoglyphics and the Determination of Twin Zygosity

Previous Serial Number: Same

Principal Investigators: Chris C. Plato, M.S., Geneticist
J. Theodore Schwartz, M.D., National Eye Institute
J. D. Niswander, D.D.S., National Institute of Dental Research

Other Investigators: None

Cooperating Unit: Field and Developmental Research, National Eye Institute

Man Years

Total	:	0.35
Professional	:	0.05
Other	:	0.30

Project Description:

Objectives: This study will attempt to establish criteria for the determination of zygosity in twins from the dermatoglyphic data.

Methods Employed: Finger and palm prints are taken from each pair of twins of known zygosity from blood typing. The study will involve over 500 pairs of twins participating in the ophthalmologic investigations conducted by Dr. J. T. Schwartz (NEI(CF)-70E001). The following dermatoglyphic aspects will be studied in detail: fingerprint patterns, main line terminations, presence of interdigital patterns, amplitude of the atd angle, presence of accessory triradii and presence of simian crease.

Major Findings: Project is still in progress.

Significance to Biomedical Research and the Program of the Institute: Knowledge of the extent of congruency of dermatoglyphics in monozygotic and dizygotic twins may permit reduction of the probability of error involved in the diagnosis of zygosity using blood typing alone.

Proposed Course of Project: The collected prints are presently being evaluated.
Honors and Awards: none. Publications: none.

Serial No. HD-CD9(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Dermatoglyphics of the Islanders of Tongariki in New Hebrides

Previous Serial Number: Same

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigator: D. Carleton Gajdusek, M.D., Special Chronic Disease Studies, Collaborative and Field Research, National Institute of Neurological Diseases and Stroke

Cooperating Unit: Special Chronic Disease Studies, Collaborative and Field Research, National Institute of Neurological Diseases and Stroke

Man Years

Total	:	0.05
Professional	:	0.05
Other	:	0.00

Project Description: Complete

Honors and Awards: None

Publications: Plato, C.C., and Gajdusek, C. D.: Dermatoglyphics of the Natives of Tongariki in the New Hebrides. Hum. Biol. in Ocean., 1: 90-96, 1971.

Serial No. HD-MR9(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Cytogenetic Evaluation of Children with Congenital Anomalies and Mental Retardation

Previous Serial Number: Same

Principal Investigator: Matti S. Al-Aish, Ph.D., Research Cytogeneticist

Other Investigators: None

Cooperating Units: Departments of Pediatrics and Endocrinology, National Naval Medical Center, U.S. Naval Hospital, Bethesda, Maryland
DeWitt Army Hospital, Andrew Rader Army Clinic, Fort Myer, Andrews Air Force Base
Hospital for Sick Children, Washington, D.C.

Man Years

Total	:	0.10
Professional	:	0.10
Other	:	0.00

Project Description: Complete

Honors and Awards: None.

Publications: Bachman, R., de la Cruz, F., Al-Aish, M.S., and Santell, F.: Short arm deletion of an X chromosome in a 19 year old girl. Amer. J. of Ment. Defic. 75:435-441,1971.
Al-Aish, M.S., Dodson, W. E., and Plato, C.C. Down's Syndrome with XYY: 48,XYY,G+. Amer. J. of Dis. of Child. 121:444-446,1971.

Serial No. HD-MR10/11(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Morphology Studies on Normal and Abnormal Human Chromosomes

Previous Serial Number: Same

Principal Investigator: Matti S. Al-Aish, Ph.D., Research Cytogeneticist

Other Investigators: None

Cooperating Units: National Naval Medical Center, Bethesda, Maryland

Man Years

Total	:	0.40
Professional	:	0.40
Other	:	0.00

Project Description: Complete.

Honors and Awards: None

Publications: Al-Aish, M.: Human chromosome morphology. III. Pronase effects on chromosome structure in vitro. Cytologia 36:539-542, 1971. Al-Aish, M.: Human Cytogenetics. In Brown, W.V.: Textbook of Cytogenetics. St. Louis, Mo., C.V.Mosby, in press.

Serial No. HD-CD10(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Dermatoglyphic of the Kukukuku and Fore Peoples of the Kuru Region of New Guinea

Previous Serial Number: Same

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigators: Carlton Gajdusek, M.D.
Special Chronic Disease Studies
Richard Sorenson, M.S.
Special Chronic Disease Studies

Cooperating Unit: Special Chronic Disease Studies, Collaborative and Field Research, National Institute of Neurological Diseases and Stroke

Man Years

Total	:	0.02
Professional	:	0.02
Other	:	0.00

Project Description: Complete

Honors and Awards: Results presented at the Annual Meeting of the American Association of Physical Anthropologists, Boston, Massachusetts, April 1971.

Publications: Plato, C. and Gajdusek, C.: Genetic Studies in Relation to Kuru: V. Dermatoglyphics of populations of the Eastern Highlands of New Guinea. Am. J. Hum. Genet., in press, (part of a monograph on Kuru).

Serial No. HD-CD11(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Dermatoglyphics of the Micronesians

Previous Serial Number: Same

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigators: D. Carleton Gajdusek, M.D.
Special Chronic Disease Studies, Collaborative and Field Research, National Institute of Neuroligical Diseases and Stroke
Paul Brown, M.D., Collaborative and Field Research, National Institute of Neurological Diseases and Stroke

Cooperating Unit: Special Chronic Disease Studies, Collaborative and Field Research, National Institute of Neurological Diseases and Stroke

Man Years

Total	0.25
Professional	0.10
Other	0.15

Project Description:

Objectives: The purpose of this project is to further define the genetic composition of the people of Micronesia through complete dermatoglyphic studies, to review the literature pertaining to the worldwide distribution of various dermatoglyphic patterns, and to assess the significance of dermatoglyphics as a criterion for genetic relationship among different populations.

Methods Employed: The dermatoglyphic data were collected in conjunction with previous serological and epidemiological investigations in the area. Eight hundred sets of finger and palm prints from all the atolls of the district of Yap are presently being analyzed for the determination of the frequencies of the various finger and palm patterns and configurations.

Major Findings: This project is still in progress.

Significance to Biomedical Research and the Program of the Institute: A genetic identification of a population is helpful in order to facilitate and explain some of the results of the medical and neurological surveys by establishing certain patterns of gene migration or genetic relationships.

Proposed Course of Project: All the prints have been collected and evaluated. Statistical analysis of data is completed.

Honors and Awards: None

Publications: Plato, C.C., Brown, P. and Gajdusek, D. C.: Dermatoglyphics of various micronesian populations: with notes for modification of the palmar dermatoglyphic nomeclature. Presented at the IV international Congress of Human Genetics and the III International Conference on dermatoglyphics. Book of Abstracts. Pp. 201 September 6-11, 1971, Paris, France.

Plato, C. C., Brown, P. and Gajdusek, D. C.: Dermatoglyphics of the Micronesians from the Outer Islands of Yap. Zeitscrift fur Morph. und. Anthrop. 1972. In press.

Serial No. HD-CD14(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Psoject Report
July 1, 1971 through June 30, 1972

Project Title: The Role of Xg^a Incompatibility in Fetal Loss: Family Studies

Previous Serial Number: Same

Principal Investigators: Chris C. Plato, M.S., Geneticist
Felix de la Cruz, M.D., M.P.H.
Ronald Bachman, M.D., Permanente Medical Group, Oakland, California

Other Investigators: None

Cooperating Unit: Permanente Medical Group, Oakland, California

Man Years

Total	:	0.05
Professional	:	0.02
Other	:	0.03

Project Description:

Objectives: The aims of this project are (1) to confirm through family studies our earlier observations that Xg^a incompatibility may be responsible for the diminution of the expected frequency of female births to Xg^a negative mothers, and (2) to establish the degree of difference between the expected and observed frequency in female births (selection coefficient).

In our previous report, "Possible Intrauterine Interaction Between Twins Incompatible for the Xg^a Blood Group," we found a deficiency of female Xg^a positive twins. This observation held true in both the monozygotic as well as the dizygotic twins. On the other hand the observed numbers of each type of male twins were very similar to what one would expect for a sex-linked gene under random mating (no selection). Our hypothesis was that this loss of female twins is a result of mother-daughter incompatibility for this blood group which occurs when the mother is Xg^a negative ($Xg^{a-}Xg^{a-}$) and the father Xg^a positive ($Xg^{a+}Y$) and the resulting dauther Xg^a heterozygotic positive ($Xg^{a+}Xg^{a-}$). This explanation agrees very well with the results of a study by Mann et al. (in press), who arrived at the same conclusion by studying the sex ratio. The results of the aforementioned studies are based on

population expectations using the established gene frequencies of p = .65 and q = .35 for the Xg^{a+} and the Xg^{a-} alleles respectively.

Methods Employed: The present investigation will study the segregation of the Xg^a blood group in sibships. Blood was collected from parents and all the siblings including abortuses whenever possible. Our data were obtained from the Permanente Medical Group in Oakland.

Major Findings: Five hundred expectant mothers have been screened for the Xg^a blood group. Of these 65 were Xg^{a-}, and 50 Xg^a weak positive. These 115 individuals plus 75 Xg^a positive controls formed the basic sample for family studies. Blood samples were collected from a number of the spouses. The outcome of each pregnancy was obtained and the data are presently being evaluated.

Significance to Biomedical Research and the Program of the Institute: The importance of this type of study to medical and child health research has been demonstrated in our earlier twin study (HD-CD16(c)). Xg^a incompatibility may be a significant factor in fetal loss, affecting only the female offspring.

Proposed Course of Project: Analysis of the data and evaluation of the result are in progress.

Honors and Awards: None

Publications: None

Serial No. HD-CD21(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Eidetic Imagery in Brain-Injured Children

Previous Serial Number: Same

Principal Investigator: Jean S. Symmes, Ph.D., Research Psychologist

Other Investigators: None

Cooperating Units: Christ Church Child Center, Bethesda, Maryland
St. Maurice's School, Potomac, Maryland

Man Years

Total	:	0.00
Professional	:	0.00
Other	:	0.00

Project Description: Complete

Honors and Awards: Paper read at the American Psychological Association meetings, September 1971.

Publications: Symmes, J. S.: Visual imagery in brain-injured children. Percept. Mot. Skills 33:507-514, 1971.

Serial No. HD-CD24(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Reading Inefficiency: Perceptual Studies

Previous Serial Number: Same

Principal Investigator: Jean S. Symmes, Ph.D., Research Psychologist

Other Investigators: Judith Rapoport, M.D., Department of Psychiatry, Georgetown University

Cooperating Units: None

Man Years

Total	:	0.0
Professional	:	0.0
Other	:	0.0

Project Description: Complete

Honors and Awards: None

Publications: Symmes, J. S. and Rapoport, J. L.: Unexpected reading failure. Amer. J. Orthopsychiat. 42:82-91, 1972.

Serial No. HD-CD28(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Lipid Metabolism in Down's Syndrome

Previous Serial Number: Same

Principal Investigators: Ira T. Lott, M.D., Clinical Associate
Edwin H. Kolodny, M.D., Research Associate, National Institute of Neurological Diseases and Stroke
Roscoe O. Brady, M.D., Chief, Section on Lipid Chemistry, Laboratory of Neurochemistry, National Institute of Neurological Diseases and Stroke

Other Investigators: Matti S. Al-Aish, Ph.D., Research Cytogeneticist

Cooperating Unit: Laboratory of Neurochemistry, Section on Lipid Chemistry, National Institute of Neurological Diseases and Stroke

Man Years

Total	:	0.00
Professional	:	0.00
Other	:	0.00

Project Description: Complete

Honors and Awards: None

Publications: None

Serial No. HD-CD29(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Circadian Periodicity in a Child with the Prader-Willi Syndrome

Previous Serial Number: Same

Principal Investigator: Ira T. Lott, M.D., Clinical Associate

Other Investigators: David Bailey, M.D., Medical Officer, National Naval Medical Center
Ralph D. Feigin, M.D., Chief, Infectious Disease Service, St. Louis Children's Hospital, St. Louis, Missouri

Cooperating Units: Pediatric Service, National Naval Medical Center, Bethesda, Maryland
The Edward Mallinckrodt Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri

Man Years

Total	:	0.00
Professional	:	0.00
Other	:	0.00

Project Description: Complete

Honors and Awards: None

Publications: None

Serial No. HD-CD30(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Blood Lactic Acid Metabolism and Urinary Catecholamines in Hyperactive Children with High Anxiety Ratings

Previous Serial Number: Same

Principal Investigators: Ira T. Lott, M.D., Clinical Associate
Duane Alexander, M.D., Assistant to Scientific Director

Other Investigators: Judith Rapoport, M.D., Department of Psychiatry, Georgetown University
Alice Abramson, M.S., Education Specialist

Cooperating Units: None

Man Years

Total	:	0.0
Professional	:	0.0
Other	:	0.0

Project Description: Complete

Honors and Awards: None

Publications: Rapoport, J., Abramson, A., Alexander, D., and Lott, I.: Playroom observations of hyperactive children on medication. J. Amer. Acad. Child Psychiat., 10, 524-534, 1971.

Serial No. HD-CD35(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Use of Twin Studies to Investigate the Lyon Hypothesis of X Chromosomal Inactivation with the Xg Blood Group System as Marker

Previous Serial Number: Same

Principal Investigators: Chris C. Plato, M.S., Geneticist
J. Theodore Schwartz, M.D., National Eye Institute
Joseph D. Mann, M.D., Butterworth Hospital

Other Investigators: None

Cooperating Units: Field and Developmental Research, National Eye Institute. Department of Pathology, Butterworth Hospital, Grand Rapids, Michigan

Man Years

Total	:	0.20
Professional	:	0.05
Other	:	0.15

Project Description:

Objectives: This study will attempt to provide information pertinent to the Lyon hypothesis of X chromosome inactivation.

Methods Employed: Fifteen pairs of monozygotic twins have been identified as discordant for the Xg blood type. Blood specimens were obtained from them and their parents and sibs for typing by standard methods.

Major Findings: Evaluation of the results is still in progress.

Significance to Biomedical Research and the Program of the Institute: Study of the Xg blood group marker in identical twins can provide supporting or refuting evidence for the Lyon hypothesis.

Proposed Course of Project: Data have been collected and are presently being evaluated.

Honors and Awards: None

Publications: None

Serial No. HD-CD36(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Dermatoglyphics of a Native Population of West New Guinea

Previous Serial Number: Same

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigator: Carleton Gajdusek, M.D.
Special Chronic Disease Studies, National Institute of Neurological Diseases and Stroke

Cooperating Units: Special Chronic Disease Studies, Collaborative and Field Research, National Institute of Neurological Diseases and Stroke

Man Years

Total	:	0.10
Professional	:	0.05
Other	:	0.05

Project Description: Complete

Honors and Awards: None

Publications: Plato, C. C. and Gajdusek, C.: Dermatoglyphics of the Natives Western New Guinea; Human Biology in Oceania: In press.

Serial No. HD-CD 38
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Glycoprotein Metabolism in Pineal Glands in Vitro

Previous Serial Number: None

Principal Investigator: Ira T. Lott, M.D., Clinical Associate

Other Investigators: Richard H. Quarles, Ph.D., Senior Staff Fellow, Laboratory of Neurochemistry, Section on Lipid Chemistry, National Institute of Neurological Diseases and Stroke
David C. Klein, Ph.D., Senior Staff Fellow, Section on Physiological Controls, Laboratory of Biomedical Sciences, National Institute of Child Health and Human Development

Cooperating Units: Laboratory of Neurochemistry, National Institute of Neurological Diseases and Stroke
Laboratory of Biomedical Sciences, National Institute of Child Health and Human Development

Man Years

Total	:	0.00
Professional	:	0.00
Other	:	0.00

Project Description: Complete

Honors and Awards: Paper presented by I. T. Lott at the 1971 meeting of the Fed. Amer. Soc. Exp. Biol., Chicago.

Publications: Lott, I. T., Quarles, R. H., Klein, D. C. Glycoproteins are synthesized and secreted by pineal glands in vitro. Proc. Fed. Amer. Soc. Exp. Biol., 30:364, 1971.

Lott, I. T., Quarles, R. H., and Klein, D. C. Biosynthesis and release of glycoproteins by pineal gland in organ culture. Biochem. Biophys. Acta 264:144-151, 1972.

Serial No. HD-CD39(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Australia Antigen in Various Types of Down's Syndrome

Previous Serial Number: None

Principal Investigator: Matti S. Al-Aish, Ph.D., Research Cytogeneticist

Other Investigators: W. E. Dodson, M.D., Children's Hospital, St. Louis, Mo.
Earl Matthew, M.D.
Dale E. Dietzman, M.D.
John L. Sever, M.D.
National Institute of Neurological Diseases and Stroke

Cooperating Unit: Perinatal Research Branch, National Institute of Neurological Diseases and Stroke

Man Years

Total	:	0.00
Professional	:	0.00
Other	:	0.00

Project Description: Complete.

Honors and Awards: None

Publications: Matthew, E. B., Dietzman, D. E., Madden, D. L., Sever, J. L., Al-Aish, M.S., and Dodson, W.E.: Possible absence of Australia antigen in G/G translocation type of Down's syndrome. Lancet:I,702,1971.

Serial No. HD-CD40(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Cytogenetic Studies on Long Term Lymphocyte Cultures of Cystinotics, Carriers, and Normal Subjects

Previous Serial Number: None

Principal Investigator: Matti S. Al-Aish, Ph.D., Research Cytogeneticist
W. W. Dodson, M.D., Children's Hospital, St. Louis, Mo.

Other Investigators: J. D. Schulman, M.D.
K. H. Bradley
I. K. Berezesdy
National Institute of Arthritis and Metabolic Diseases
P.M. Grimley, M.D., National Cancer Institute

Cooperating Units: National Institute of Arthritis and Metabolic Diseases
National Cancer Institute

Man Years

Total	:	0.00
Professional	:	0.00
Other	:	0.00

Project Description: Complete

Honors and Awards: None

Publications: Schulman, J.D., Bradley, K.H., Berezesky, I.K., Grimley, P.M., Dodson, W.E., and Al-Aish, M.S.: Biochemical morphologic, and cytogenetic studies of leukocytes growing in continuous cultures from normal individuals and patients with cystinosis. Ped. Res. 5:501-510,1971.

Serial No. HD-CD41(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Structure and Color of the Iris in 250 Pairs of Twins

Previous Serial Number: Same

Principal Investigators: Chris C. Plato, M.S., Geneticist
J. Theodore Schwartz, M.D., National Eye Institute

Other Investigators: None

Cooperating Unit: Field and Developmental Research, National Eye Institute

Man Years

Total	:	0.15
Professional	:	0.05
Other	:	0.10

Project Description:

Objectives: (1) To establish possible association between iris structure and color and several congenital eye anomalies, and (2) to establish a scale for iris color and estimate its heretability.

Methods Employed: All of the twin pairs have undergone a thorough eye examination and evaluations. Their irises were photographed and slides were made and mounted in pairs. These slides will be used for iris structure studies. In view of technical color distortions involved in photography and slide preparation, pigment evaluation will have to be made directly by comparing the iris of each (live) eye to a predetermined set of eye color and shade model scale composed of artificial eyes.

Major Findings: Project is still in progress.

Significance to Biomedical Research and the Program of the Institute: 1) To establish possible associations between iris structure and several eye disorders, 2) to study the heretability of iris pigment and its possible relationship with eye anomalies, and 3) to offer additional criteria for the establishment of zygocity among twins.

Proposed Course of Project: To evaluate the structure and pigment of the irises and study their relationship with eye anomalies.

Honors and Awards: None

Publications: None

Serial No. HD-CD42(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Association Between Bilateral Asymmetry in Dermatoglyphics and Handedness

Previous Serial Number: Same

Principal Investigators: Chris C. Plato, M.S., Geneticist
Wladimir Wertelecki, Medical University of South Carolina

Other Investigators: None

Cooperating Unit: Medical University of South Carolina

Man Years

Total	:	0.15
Professional	:	0.05
Other	:	0.10

Project Description:

Objectives: To establish the effect of handedness upon the bilateral asymmetry in dermatoglyphics and obtain better ideas about the embryological factors influencing both of these traits.

Methods Employed: Dermatoglyphics of two hundred left handed individuals and an equal number of individually matched right handed controls have been collected by standard ink method.

Major Findings: The project is data evaluation stage.

Significance to Biomedical Research and the Program of the Institute: To understand better the embryological factors influencing the formation of dermatoglyphics and their consequent associations with clinical anomalies.

Proposed Course of Project: To finish evaluation of the data.

Honors and Awards: None

Publications: None

Serial No. HD-CD43(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Dermatoglyphic Features of Normal Caucasian Americans

Previous Serial Number: Same

Principal Investigators: Chris C. Plato, M.S., Geneticist
Florence Steinberg, M.S., Head, Analytical Unit, Program Statistics Branch
James Cereghino, M.D., Collaborative and Field Research, National Institute of Neurological Diseases and Stroke

Other Investigators: Bernard Fox, M.D., Perinatal Research Branch, National Institute of Neurological Diseases and Stroke
James Drorbaugh, M.D., Children's Hospital Medical Center, Boston, Massachusetts

Cooperating Units: Analytical Unit, Program Statistics Branch, National Institute of Child Health and Human Development
Collaborative and Field Research, National Institute of Neurological Diseases and Stroke
Children's Hospital Medical Center, Boston, Massachusetts

Man Years

Total	:	0.40
Professional	:	0.15
Other	:	0.25

Project Description:

Objectives: To collect dermatoglyphics (finger and palm prints) from a large sample of normal Caucasian children. The use of such a sample would be twofold: 1) To provide dermatoglyphic data from normal individuals to serve as controls for our ongoing studies pertaining to the dermatoglyphics of certain congenital anomalies, and 2) to provide the frequencies of various dermatoglyphic features in a large sample of Caucasian Americans which may serve as additional genetic markers.

Methods Employed: Finger and palm prints are being collected from 1000 children seen for a seven year evaluation at the Boston Children's Hospital

in collaboration with the Perinatal Research Branch study. Prints will be calculated by the Faurot inkless method.

Major Findings: Data collection and evaluation is still in progress.

Significance to Biomedical Research and the Program of the Institute: To provide control data for comparing the dermatoglyphics of patients with various congenital anomalies.

Proposed Course of Project: To finish collection and analysis of data.

Honors and Awards: None

Publications: None

Serial No. HD-CD44(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Dermatoglyphic Features of Normal Black Americans

Previous Serial Number: Same

Principal Investigators: Florence Steinberg, M.S., Head, Analytical Unit, Program Statistics Branch
James Cereghino, M.D., Collaborative and Field Research, National Institute of Neurological Diseases and Stroke
Chris C. Plato, M.S., Geneticist

Other Investigator: Dr. Jane Hardy, Johns Hopkins University

Cooperating Units: Collaborative and Field Research, National Institute of Neurological Diseases and Stroke
Johns Hopkins University, Baltimore, Maryland

Man Years

Total	0.25
Professional	0.05
Other	0.20

Project Description:

Objectives: To collect and evaluate the dermatoglyphics of a large sample of Black Americans. Such a sample will be used as controls for our ongoing studies on the dermatoglyphics of various congenital anomalies.

Methods Employed: Finger and palm prints were collected from over five hundred Black Americans from the Baltimore area through the standard ink method.

Major Findings: Data are presently being prepared for final publication.

Significance to Biomedical Research and the Program of the Institute: To offer appropriate controls for the study of the dermatoglyphics and their developmental involvement in several congenital anomalies.

Proposed Course of Project: To prepare a manuscript for final publication.

Honors and Awards: None

Publications: Steinberg, F. S., Gereghino, J. J. and Plato, C. C.: Dermatoglyphic features of Black Americans. Presented at the IV International Congress of Human Genetics. Book of Abstracts Pp. 204. Paris, France 1971.

Serial No. HD-CD45(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Dermatoglyphics of Leukemia Patients and Their Relatives

Previous Serial Number: Same

Principal Investigators: Chris C. Plato, M.S., Geneticist
Wladimir Wertelecki, M.D., Department of Pediatrics, University of South Carolina

Other Investigators: Jerry D. Niswander, D.D.S., Department of Human Genetics, National Institute of Dental Research
J. F. Fraumeni, Epidemiology Branch, National Cancer Institute

Cooperating Units: Department of Pediatrics, University of South Carolina
Epidemiology Branch, National Cancer Institute
Human Genetics Branch, National Institute of Dental Research

Man Years

Total	:	0.35
Professional	:	0.25
Other	:	0.10

Project Description:

Objectives: This study represents a follow-up effort to establish possible associations between known dermatoglyphic markers and the various forms of leukemia.

Methods Employed: Dermatoglyphics (finger and palm prints) have been collected from 130 leukemia patients and as many of their first degree relatives as possible. All prints have been read and evaluated completely. A new computer program has been developed by the authors for data storage, retrieval and analysis.

Major Findings: An excess of whorls among the leukemia male patients. This agrees with the differences observed in the birth weight of the male patients. This becomes significant since it might relate to differences in the develop-

mental stage of leukemia between the male and female patients. The patients of both sexes showed higher frequencies of simian and Sydney palm lines.

Significance to Biomedical Research and the Program of the Institute: Demonstration of dermatoglyphic markers in leukemia would add a new tool to study of this disease.

Proposed Course of Project: Data on the families is being programmed for computer analysis. A report will be prepared for publication.

Honors and Awards: None

Publications: Wertelecki, W., Plato, C. C., Fraumeni, J. F. and Niswander, J.D.: Dermatoglyphics of patients with acute lymphatic leukemia and their first degree relatives. Presented at the IV International Congress of Human Genetics. Book of Abstracts Pp. 205. Paris, France 1971.

Serial No. HD-CD46(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Dermatoglyphics of the Natives of Papua, New Guinea

Previous Serial Number: Same

Principal Investigators: Chris C. Plato, M.S., Geneticist
Carlton Gajdusek, M.D., National Institute of Neurological Disesass and Stroke

Other Investigators: None

Cooperating Unit: Special Chronic Disease Studies, National Institute of Neurological Diseases and Stroke

Man Years

Total	:	0.10
Professional	:	0.05
Other	:	0.05

Project Description:

Objectives: To evaluate the dermatoglyphics of these people as a part of a multidisciplinary investigation to identify the frequencies of the various genetic markers of the aboriginal populations through the world.

Methods Employed: Collect finger and palm prints from three areas of Papua and evaluate them in detail.

Significance to Biomedical Research and the Program of the Institute: The study of genetic markers of the few remaining primitive populations will allow scientists later on to evaluate the effects of western culture and environmental pollution upon these societies.

Proposed Course of Project: To evaluate the collected data.

Honors and Awards: None

Publications: None

Serial No. HD-CD47(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Down's Syndrome: Cerebral Monoamine Turnover in Patients with Diminished Platelet Serotonin

Previous Serial Number: None

Principal Investigator: Ira T. Lott, M.D., Clinical Associate

Other Investigators: Dennis L. Murphy, M.D., Head, Section on Psychiatry Laboratory of Clinical Science, National Institute of Mental Health
Thomas N. Chase, M.D., Chief, Section on Experimental Therapeutics, Laboratory of Clinical Science, National Institute of Mental Health
Matti S. Al-Aish, Ph.D., Research Cytogeneticist

Cooperating Units: Laboratory of Clinical Science, National Institute of Mental Health
2-West Neuromedical Service, Clinical Center

Man Years

Total	:	0.00
Professional	:	0.00
Other	:	0.00

Project Description: Complete

Honors and Awards: Abstract presented at the 23rd Annual Meeting of the American Academy of Neurology, New York City, May 1, 1971. I. T. Lott, D. L. Murphy, and T. N. Chase. "Down's Syndrome: Cerebral monoamine turnover in patients with diminished platelet serotonin."

Publications: Lott, I. T., Murphy, D. L., and Chase, T. N.: Down's Syndrome: Cerebral monoamine turnover in patients with diminished platelet serotonin. Pediat. Res., in press.

Serial No. HD-CD48(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Dermatoglyphics and Cleft Palate: Familial Investigation

Previous Serial Number: Same

Principal Investigators: Chris C. Plato, M. S., Geneticist
J. D. Niswander, D.D.S., National Institute of Dental Research

Other Investigators: Wladimir Wertelecki, M. D., Department of Pediatrics, University of South Carolina

Cooperating Units: Human Genetics Branch, National Institute of Dental Research
Department of Pediatrics, University of South Carolina

Man Years

Total	:	0.35
Professional	:	0.20
Other	:	0.15

Project Description:

Objectives: This study will look for possible associations between dermatoglyphic markers and the various forms of cleft palate.

Methods Employed: Finger and palm prints were collected from cleft palate patients, their parents, and sibs from over 100 families. An equal number individually matched controls were selected and their dermatoglyphics were also obtained along with those of their first degree relatives. The results will be statistically compared for possible differences between (1) individual patients and controls, (2) patients and their first degree relatives, (3) the patient families and controls, and (4) bilateral differences will also be investigated.

Major Finding: The project is still in progress.

Significance to Biomedical Research and the Program of the Institute: Identification of dermatoglyphic markers associated with cleft palate would be of possible assistance in understanding the genetics and origin of this malformation.

Proposed Course of Project: The dermatoglyphics of all of the controls and their relatives have been completed. Most of the patient data have also been evaluated.

Honors and Awards: None

Publications: None

Serial No. HD-CD49(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Dermatoglyphics of Patients with Congenital Heart Disease

Previous Serial Number: Same

Principal Investigators: Chris C. Plato, M.S., Geneticist
Wladimir Wertelecki, M.D., Medical University of South Carolina

Other Investigators: None

Cooperating Unit: Medical University of South Carolina

Man Years

Total	: 0.15
Professional	: 0.10
Other	: 0.05

Project Description:

Objectives: To establish possible associations between various dermatoglyphic traits and congenital heart disease.

Methods Employed: Finger and palm prints were collected by the standard ink method from eighty patients with congenital heart disease and are presently being evaluated.

Major Findings: Project in progress.

Significance to Biomedical Research and the Program of the Institute: To provide possible dermatoglyphic markers in an effort to understand the etiology and possible inheritance of this disease.

Proposed Course of Project: To evaluate and analyze the data. If warranted family data will be collected.

Honors and Awards: None

Publications: None

Serial No. HD-CD17(c)

1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Dermatoglyphic Evaluation of a Group of Mentally Retarded Children

Previous Serial Number: HD-CD17(c)

Principal Investigator: Chris C. Plato, M.S., Geneticist

Other Investigators: None

Cooperating Units: None

Man Years

Total	:	0.20
Professional	:	0.10
Other	:	0.10

Project Description:

Objectives: This represents a routine study of the dermatoglyphics of the patients studied at the Children's Diagnostic and Study Branch.

Methods Employed: Dermatoglyphics were collected by standard methods and are presently being evaluated. The patients were separated by degree of intelligence and the dermatoglyphic data will be evaluated accordingly.

Major Findings: Study is still in progress.

Significance to Biomedical Research and the Program of the Institute: It is a further effort to establish possible associations or genetic linkage between known genetic markers and the various types of mental deficiency.

Proposed Course of Project: Data are presently being evaluated.

Honors and Awards: None

Publications: None

Serial No. HD-CD50(c)
1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Dermatoglyphics of Certain Coastal and Mountain Tribes of Peru Indians.

Previous Serial Number: New Project

Principal Investigator: Chris C. Plato, M. S., Geneticist

Other Investigators: Ralph Garruto, Ph.D., Pennsylvania State University

Cooperating Units: Department of Anthropology. Pennsylvania State University, University Park, Pennsylvania

Man Years:

Total	:	0.15
Professional	:	0.05
Other	:	0.10

Project Description:

Objectives: This represents another of our efforts to evaluate the clinical, anthropological and genetic markers of some of the remaining aboriginal populations prior to their complete acceptance of "western" cultural, medical and pharmaceutical practices.

Methods Employed: Standard dermatoglyphic methods. They were conducted in conjunction with other serological, clinical and anthropological surveys.

Major Findings: Study in progress.

Significance to Biomedical Research and the Program of the Institute: To help us understand the progress of new diseases in isolates.

Proposed Course of Project: In evaluate the collected data.

Honors and Awards: None

Publications: None

Serial No. HD-CD51(c)

1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Palmar dermatoglyphics of Down's Syndrome: Revisited.

Previous Serial Number: New Project

Principal Investigator: Chris C. Plato, M. S., Geneticist

Other Investigators: James Cereghino, M.D., Collaborative and Field Research NINDS
Florence Steinberg M.S., Head, Analytical Unit, Program Statistics Branch, NICHD

Cooperating Units: Collaborative and Field Research, NINDS

Man Years

Total	:	0.30
Professional	:	0.25
Other	:	0.05

Project Description:

Objectives: To ascertain the usefulness of our newly established method for classifying palmar dermatoglyphic patterns in the study of Down's Syndrome.

Methods Employed: Prints were collected by standard ink method and evaluated by the method of Plato and Wertelecki. (Serial No.)

Major Findings: Using the new classifications we were able to establish additional significant differences between the palmar dermatoglyphics of patients with Down's Syndrome and normal controls.

Significance to Biomedical Research and the Program of the Institute: A new method was shown to be of value in studying the associations of known genetic markers and congenital anomalies.

Honors and Awards: None

Publications: Plato, C. C., Cereghino, J. and Steinberg, F. S.: Palmar dermatoglyphics of Down's Syndrome: Revisited. Submitted for publication.

Serial No. HD-CD52(c)

1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: A Method for Subclassifying the Interdigital Patterns: A Comparative Study of the Palmar Configurations.

Previous Serial Number: New Project:

Principal Investigator: Chris C. Plato, M. S., Geneticist

Other Investigators: Wladimir Wertelecki, M.D.

Cooperating Units: Department of Pediatrics, University of South Carolina

Man Years

Total	:	0.25
Professional	:	0.25
Other	:	0.00

Project Description:

Objectives: To establish a detailed method for classifying the palmar dermatoglyphic patterns. Such a method would make population and clinical dermatoglyphic comparisons more meaningful.

Methods Employed: The new classification for palmar patterns was formulated by taking into consideration the direction of the main line and the presence of an accessory triradius in a specific palmar area.

Major Findings: The suggested new classification offers another excellent tool for studying population as well as clinical dermatoglyphics. The various classifications showed significant differences among various populations as well as among congenital anomalies.

Significance to Biomedical Research and the Program of the Institute: It offers another valuable tool in establishing associations between known genetic markers and various congenital anomalies.

Propose Course of Project: Project completed.

Honors and Awards: None

Publications: Plato, C. C., and Wertelecki, W.: A method for subclassifying the Interdigital patterns: A comparative study of the palmar configurations. Am. J. Phys. Anthropology. (In press.)

Presented at the IV International Congress of Human Genetics and the III International conference of dermatoglyphics. September 6-11, 1971, Paris France. Book of Abstracts Pp. 201.

Serial No. HD-CD53(c)

1. Children's Diagnostic and Study Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Analysis of Dermatoglyphic Data by Digital Computer

Previous Serial Number: New Project

Principal Investigators: Chris C. Plato, M. S., Geneticist,
Wladimir Wertelecki, M. D., University of South Carolina

Other Investigators: None

Cooperating Units: Department of Pediatrics, University of South Carolina Medical School, Charleston, South Carolina

Man Years

Total	:	0.15
Professional	:	0.15
Other	:	0.00

Project Description:

Objectives: To develop a fast and inexpensive method for evaluating population, clinical and familial dermatoglyphic data.

Methods Employed: Time sharing systems and IBM computers.

Major Findings: Three different methods were developed which can be utilized depending on the needs of the investigator. Special forms for recording the raw and final data were also developed.

Significance to Biomedical Research and the Program of the Institute: It offered speedy, accurate and relatively inexpensive methods for evaluating dermatoglyphic data. It also provides for storage and retrieval of the data for future use.

Proposed Course of Project: Project Completed

Awards and Honors: None

Publications: Wertelecki, W. and Plato, C. C.: Analysis of dermatoglyphic data by digital computer. Presented at the IV International Congress of Human Genetics and the III International conference of dermatoglyphics. September 6-11, 1971, Paris, France. Book of Abstracts Pp. 205.

NICHD Annual Report
July 1, 1971 through June 30, 1972

Gerontology Research Center
Office of the Chief

Highlights

1. Nutritional deficiencies frequently seen in the aged are usually ascribed to the low incomes often found in the elderly. However, a relatively high incidence of deficiencies in vitamins of the B complex has been found in tests made on 200 community residing males from the upper middle class--subjects in whom income was not a limiting factor in their diets. Twenty percent of the group who were not taking vitamin supplements had abnormally low levels of thiamine (B_1) and riboflavin (B_2) in their blood and 50% were deficient in pyridoxine (B_6). In contrast, subjects taking vitamin supplements showed normal vitamin levels.

These results indicate that nutritional deficiencies may be more prevalent among the elderly than has been believed and that such deficiencies can be avoided.

2. It has been shown that reaction time does not necessarily decline with age. Some of the factors which may contribute to an age effect, but which are not part of the reaction time task per se, are: The intertrial intervals (very short intervals may have a more severe effect upon the elderly than upon the young); fatigue (a great number of trials may affect the elderly more than the young); previous experience (older persons who have worked with their hands may be less likely to show slower reaction times than are young persons).

3. No differences in reasoning behavior were found between old and young men when they were matched in terms of non-verbal intelligence. However, among unmatched samples there was a highly significant age effect--the older men were poorer performers than were the younger men. The major difference between the two studies was that the test of non-verbal intelligence tends to eliminate more older men than younger men. These data show that old men are just as capable as young men in reasoning ability; however, the data also underscore the need to learn why some older subjects decline in intelligence while others do not.

4. Longitudinal analyses of tests of serial learning and of associative learning have shown that learning ability under pacing conditions declines with age; as subjects age they make increasingly more errors when the material to be learned is presented at a relatively high rate. Detailed analyses of the data show that learning ability does not decline with age under slow pacing, except among subjects in their 70s. This finding indicates that the age-related decline in performance among the older men

cannot be attributed to learning ability, per se, but rather to the deleterious effects of time pressure. Further analyses of the errors made by the old men under time pressure show that the errors these men made were errors of omission--the men were unable to respond correctly and, therefore, tended not to respond at all. Errors of commission were not different among the age groups. In fact, under some conditions these errors decreased longitudinally among all age groups except the oldest (subjects in their 70s).

5. Studies of the effect of task complexity and learning have shown that performance declines with age. Subjects were confronted with a task which required them to learn three lettered words in which the letters were misarranged. After the subjects had learned the words, they were required to reconstitute the original misarranged word according to various sets of rules. It was observed that as the rules became less specific (e.g., "rearrange the letters to make the original word") the older subjects made more errors than the younger subjects. When the rules were quite specific (e.g., "reverse the first two letters"), the old subjects were as good as the young men. The mechanisms which underlie the coding and organization of newly acquired information seem to be fundamental to the age-related changes in learning. Further studies of information processing should help to better understand these mechanisms.

6. It is now possible, with the aid of the SAAM Computer Program, to define with confidence the size of three components of the insulin pool and the rate constants describing the exchange of insulin among these pools. Pool 1 is dimensionally the size of the plasma volume, 4.6% of body weight. Pool 2 is small, 1.9% of BW and in rapid equilibrium with Pool 1. Pool 3 is relatively large, 9.3% BW, and is in slow equilibrium with Pool 1. The total size of the 3 pools is essentially that of the extracellular space. The pattern of insulin concentration changes in Pools 2 and 3 can be calculated when only insulin concentration in Pool 1 can be directly measured. The importance of this advance is shown by the fact that the delay in the response of peripheral tissues to changes in plasma insulin concentration is apparently determined by time needed for insulin to diffuse from Pool 1 to Pool 3, which represents the interstitial fluid of the peripheral tissues. Of direct pertinance to certain aging theories is the fact that there was no demonstrable difference in pool sizes and insulin kinetics between 16 young adult and 6 aged subjects. Thus differences in carbohydrate metabolism between old and young subjects cannot be ascribed to hinderances in diffusion of insulin across the capillary barrier or through the interstitial spaces.

7. The importance of physiological control mechanisms in aging has been shown in rats. The effectiveness of the carotid sinus, which responds to a rise in blood pressure by lowering the heart rate, is reduced in aged rats, i.e., the heart rate drops more in young than in old rats with the same increment in blood pressure.

8. The capacity to carry out skeletal and cardiac muscle work declines markedly with age. Studies have provided evidence indicating a correspondence between this decrease in physiological function and the deterioration with senescence in the ability of the working tissues to meet the bio-

energetic demands during activity. In model animal systems, an age-dependent degeneration of muscle mitochondria, characterized by the deformation of the inner membranes (cristae) was found. This ultrastructural degradation was concomitant with a lowered maximally stimulatable rate of substrate oxidation and a decreased respiratory control. The locus of the defect observed in aging seems to be in the respiratory chain of enzymes that supply the energy for muscular contraction.

9. Studies using human tissue culture cells have indicated that the antiviral defense mechanisms of the cells change with age. The fibroblasts were challenged by viruses after the induction of interferon with poly (I), poly (C). It was found that antiviral defenses were induced most readily in the middle of the in vitro lifespan, and that they dropped off sharply before the cells die.

10. A decrement with age in metabolic control systems, indicated by age-dependent lags in the time required to initiate enzyme induction during adaptation to stress, has recently been proposed. This hypothesis was tested by studying the rate of induction of the liver mitochondrial enzyme, α-glycerolphosphate dehydrogenase, by thyroid hormone. In this model system, the biochemical machinery for protein synthesis in aged animals was in no way impaired, i.e., the livers of senescent animals were fully capable of responding to direct adaptive stress.

11. Studies on the aging process at the molecular level indicated that divalent metal ions induced errors in the reading of the genetic code. This mispairing during the translation mechanism could effect the incorporation of the wrong amino acids into proteins during protein synthesis, with the concomitant loss of the biological function of the protein. These investigations provide a mechanism for the production of errors in genetic translation, as has been proposed as an explanation for cellular aging.

12. In other tests to determine whether senescence is correlated with an aberration in genetic information transfer, it was found that message RNA levels, quantitated by hybridization techniques, in the blowfly decreased by a factor of at least 10 during the lifespan of the animal.

Office of the Chief

Research Services

The Technical Development Section has continued its maintenance and development services to the scientific programs at the Center. Several new instruments have been developed along with the day-to-day maintenance services and technical consultations. Advancements have been made in the capabilities of the GRC computer system.

The Section has installed and standardized equipment required for cardiac catheterization of dogs in support of new research programs initiated by the Cardiovascular Section. A cardio-contouragraph was constructed for this Section to aid in determining the number of ectopic beats in ECGs

recorded in normal human subjects during their daily activities. The Psychophysiology Section was assisted in its development of a computerized conditioning laboratory by the design of an interface between the computer and recording equipment and the construction of circuitry to allow the accurate determination of beat-to-beat systolic and diastolic blood pressures. Near completion is a system for the Human Performance Section which will record, in printed form, concentrations of O_2 and CO_2 in expired air, heart rate, blood pressure, ventilation volume, and elapsed time during and following standardized exercise. Optical calipers were constructed for the Human Performance Section which will greatly reduce the data reduction time in nerve-propagation velocity measurements. Thermostatically controlled chambers were developed for the Metabolism Section Section to heat a subject's hand, thereby increasing blood flow and thus arterializing the venous effluent. This will eliminate the need for arterial punctures in glucose-clamp experiments. A building alarm system has been completed and is currently undergoing final testing. This system will allow many laboratory functions to be monitored at night by the building guard staff.

The Center's digital computer system has been expanded both in hardware and software capabilities. The digital input/output facilities were increased by the addition of a multiplexer designed and constructed by the Technical Development Section. The plotting equipment has been enhanced by the addition of an oscilloscope which allows editing of plots before taking the time to draw them. A program has been developed for the Human Performance Section so that data tapes from the Longitudinal Study can be quickly scanned and selected data can be presented on the scope-plotter system. Two programs were developed for the Section on Human Learning and Problem Solving. A video terminal, purchased by this Section, was incorporated into a system which administers expanded problem solving tests. Data from these tests are produced on magnetic tape for easy insertion into the data files of the Longitudinal Program. The computer is also being used in an attempt to produce high quality dichotic stimulation tapes. This project requires the digital recording of audio information, which is then selectively reproduced on an audio tape system. Minor successes produced so far indicate that this can be accomplished. The Technical Development Section in collaboration with other Sections will soon be analyzing the frequency components of recordings of dog and rat ventricular blood pressures. This project will support dP/dt measurements being made in other experiments.

The Photography and Arts Section produced 350 drawings, 100 photographic negatives, 3,500 photographic prints and over 2,000 projection slides at the request of 40 GRC scientists and 5 guest scientists. In addition, technical assistance was rendered to scientists employing still photography and motion pictures within their projects. New developments in the field of visual communications are being investigated for possible integration into Gerontology programs.

Rats from the "clean" colony which was started in June 1969 are now being issued to investigators in the GRC by the Animal Resources Facility. This colony, which will provide 50 males and 50 females aged 12 and 24 months during each month, has now stabilized at a population of approximately 9,500 animals. Mortality curves have been prepared for this colony.

The 50% mortality point is 23.7 months for males and 25.3 months for females. The colony is currently free of pulmonary infections. The primary causes of death in the colony (based on gross inspection) are tumors and glomerular nephritis. The open rat colony has now been phased out. Plans have been drawn up to expand the future production of senescent rats by contracting with a commercial animal producer.

In addition to the rat colony, the ARF provides cage cleaning services to individual investigators and maintains 36 dogs, 5 rhesus monkeys, 100 rabbits, 50 guinea pigs, and 600 mice.

An automatic watering system was installed in part of the rat colony on a trial basis. It has performed well and the system will be expanded to the remainder of the rat colony. During the coming year, a system for routine pathological surveillance of the animal colony will be developed.

Information Services. Contacts with news media interested in research on aging increased from 69 last year to 118, an increase of 42%. The number of tours arranged and public inquiries answered also rose slightly, 7% and 4%, respectively.

Popular articles on Center studies appeared in well known papers such as the New York Times, the National Observer, the Philadelphia Inquirer, the Chicago American, the Washington Evening Star, the Baltimore Sun, and La Stampa (Italy). Major news magazines contacting the GRC included Life, Time, Newsweek, the New York Times Magazine, Vogue, and Reader's Digest. The Baltimore unit was mentioned in a number of special audience publications, including Medical World News, JAMA Medical News, Chemical & Engineering News, Medical Tribune, Archives of Internal Medicine, and Oasis, the employee magazine of the Social Security Administration.

Center staff were featured on two local television shows during the year. Dr. Reubin Andres was a guest on "Opportunity Line," WBAL-TV (Channel 11), and Mrs. Marlene Butler, GRC nutritionist, was interviewed on "Women's Angle," WMAR-TV (Channel 2). Investigations underway at the Center were filmed by television crews from BBC-TV (Britain), Nippon NET, Japan Broadcasting, and Bayerischer Rundfunk TV (Munich, Germany). Additional news inquiries came from KCET-TV (California), WNBC-TV (New York), WMPB-TV (Maryland), and Radio Sweden.

The GRC Information Officer managed the Physical and Mental Health Section headquarters suite for the 1971 White House Conference on Aging, and served as a resource person for a community conference on aging held in Baltimore earlier in the year.

In addition to providing the usual services to GRC investigators, the Library staff continued to search out and index the world literature relating to gerontology and geriatrics. During the year, over 3,600 references were identified and classified. The reference lists were published quarterly in the Journal of Gerontology.

Collaborative Guest Scientist Program

During the year 10 guest scientist programs were supported by the GRC. These programs were staffed by 26 investigators with 32 supporting personnel. Guest scientist programs range from studies of learning and memory in humans to investigations of morphological and physiological changes in growth, development, and aging of cells in tissue culture.

Human fibroblasts, grown in tissue culture, show changes in fine structure as seen in the electron microscope. The rough endoplasmic reticulum is more dilated in younger cells; the mitochondria from young cells have conventional morphology, and often have cristae traversing the width of the organelle with visible connections to the two opposite inner mitochondrial membranes. In older cells mitochondria assume bizarre shapes and generally have fewer cristae. In young cells the golgi apparatus is poorly developed. In older cells this organelle becomes prominant. Lysosomes, autophagic vacuoles, multivesicular bodies and dense bodies are more prominant in older cells as are lipid vacuoles, cytoplasmic microfibrils and highly lobed nuclei.

The population dynamics of the development of senescence in cells in tissue culture has been evaluated. With aging there is a reduction of the fraction of cells in the population which undergoes cell division as evidenced by a reduction in the percentage of cells which were able to take up ^{3}HTdR. Despite this decline, however, cells which took up the labeled precursor seemed to do so at the same rate irrespective of whether they were in young or old populations. The percent dividing cells (i.e., percent labeled nuclei) showed a linear decline with cell population age. The percentage of non-dividers in the population showed a logarithmic increase as a function of time. The significance of this relationship is (1) that there is an age-related decline in a specific functional capacity, i.e., cell division; (2) the clonal age of any population of cells can be accurately determined; (3) the time of death of any population, i.e., the time at which it can no longer be sub-cultivated, can now be predicted.

Analysis of the population dynamics of cultures treated with hydrocortisone showed that their prolongevity was not dependent on improved maintenance of the cells but rather on an actual increase of 30-40% in the number of cell divisions. This results from a greater percentage of cells in the hydrocortisone treated population being capable of dividing at any given time. Amounts of hydrocortisone significantly lower than 5 μg/ml were ineffective in producing this stimulation of cell division, while the effects of 100 μg/ml were similar to 5 μg/ml. A concentration of at least 0.3% (v/v) of serum in the medium was necessary for hydrocortisone to be effective while 10% serum was equivalent to 50% serum. Thus serum binding proteins do not seem to be the limiting factor. Among a variety of steroid hormones tested, hydrocortisone was best, while cortisone and progesterone were equal but less effective than hydrocortisone. A variety of other hormones tried at various concentrations were ineffective in stimulating cell division.

The involution of male sex accessory organs following castration in the rat has been used as a model for the study of senescent involution. Following castration there is a marked loss of cytoplasmic organelles in the prostate which takes place predominantly within secondary lysosomes. Biochemical studies show an increase in enzyme activities per lysosome whereas the activity for the entire organ is decreased. These results indicate a selective retention, lesser degradation or slower turnover of lysosomal enzymes than is true of structural and other enzyme proteins with involution.

With increasing age there is a deposition of lipofuscin (age pigment) in cells of the accessorysex glands in males. Systematic electron microscopical studies on rats have provided information on the mechanism of formation of these structures, which in many instances tend to occupy a considerable portion of the cytoplasm. The following steps in the development of lipofuscin have been visualized: segregation of organelles (mitochondria, endoplasmic reticulum) within membrane-bound spaces; degradation and partial digestion of organelle components; deposition of undigestable residues. Similar changes have been observed after castration, but the process occurs at an accelerated pace (about the 4th day after castration).

It may be postulated, from these results, that lipofuscin in target organs may result from a decline in androgen levels during aging. As androgens control synthesis of structural proteins and building blocks for replacement of obsolete components of cytoplasmic organelles, any decline in levels of these hormones could lead to abnormal accumulation of undigested residues of such obsolete organelles. On the other hand, it is also possible that autophagia of cytoplasmic organelles constitutes a normal mechanism for digestion of obsolete cytoplasmic components in the course of cell organelle turnover. A loss or decrease of some key lysosomal enzymes during aging might then lead to the accumulation of incompletely digested residues in the form of lipofuscin. These possibilities are being investigated in long-term experiments using rats kept at different levels of exogenous testosterone. Systematic analysis of lysosomal enzyme levels during the course of aging may serve to detect changes in activities that will relate to the second possibility mentioned above.

Primary amyloidosis, a condition which affects the cardiovascular system predominately, and is unassociated with any known pre-disposing disease, is thought by some to represent the effect of an occult plasma cell dyscrasia. Senile amyloidosis, which also tends to involve the cardiovascular system, may well represent an expression of the same process in aged individuals. The malignant plasma cell disease, multiple myeloma, is said to be complicated by amyloid infiltration in from 6-15% of cases and in many instances a cardiovascular distribution predominates.

A group of 35 myeloma patients, autopsied at Baltimore City Hospitals between 1960 and 1969, were compared to a group of 138 age-matched, non-myelomatous, control individuals. All available autopsy tissues were examined and included heart, lung, liver, spleen, kidney, endocrine organs, vascular tissue, and brain when available. The amount and distribution of

amyloid in both groups was compared.

The results suggested that the presence of multiple myeloma did not influence either the amount or extent of amyloid deposition which would have been expected as a function of age alone. On the other hand, the myeloma patients did differ from the control group in that amyloid accumulation was occasionally evident in the bone marrow and in approximately 40% of the myeloma patients studied, amyloid rimmed renal tubular casts were identified. Similar casts were not observed in the control population.

Crude enterotoxin from diarrheagenic E. coli was studied in models of diarrheal disease employing dogs, rabbits, and suckling mice. The enterotoxin was shown to contain both heat-stable and heat labile enterotoxic activity, demonstrable in each animal. The time-course of the enterotoxin effect was determined in dogs and shown to differ markedly from that of cholera toxin. Nevertheless, it was shown that E. coli enterotoxin does activate mucosal adenyl cyclase, the same enzyme involved in the secretory response to cholera toxin. Infection of dog duodenum with viable E. coli also produces fluid secretion and adenyl cyclase activation. E. coli enterotoxin appears to bind to intestinal mucosa by a different mechanism than does cholera toxin. The more uniform responsiveness of suckling mice to E. coli enterotoxin suggests an enhanced susceptibility of young animals to the material. Diarrhea due to E. coli in humans occurs predominantly among the very young. These observations provide a basis for further investigation of the age-related mechanisms determining susceptibility to E. coli diarrhea.

Changes in cell membrane potential in response to cholera toxin exposure were studied by micropuncture techniques. Young cells, in the mucosal crypts, were shown to be depolarized by toxin exposure, consistent with a secretory response, whereas older cells on the villus were not. Aging of these cells as they pass from crypt to villus tip appears to markedly alter their responsiveness to the toxin.

The common barnyard or zebra earthworm Esenia foetida has been used for studies on growth limitation and regeneration. Experimental studies have led to the hypothesis that cell proliferation and hence regeneration and normal growth in earthworms stops because the essential stem cells have reached the end of their clonal life span as appears to be true of certain strains of cells in vitro (Hayflick), in mammary gland ducts in vivo (Daniel), and in ciliates (Sonneborn). Three lines of evidence in annelids strongly support such an hypothesis though the possibility remains open that cellular proliferation is stopped by a progressively formed hierarchy of chalones. In that case, it may be that clonal life in vitro is similarly terminated. This hypothesis is being tested in tissue culture of cells derived from different locations in the earthworm.

Two new observations with respect to the effects of radiation on cell division and growth have been made. First, although irradiation sufficient to inhibit all regeneration for over a month by which time controls have finished regeneration gives very consistent results in such complete inhibitation; if the worms are kept for seven or more weeks (sometimes up to 12 weeks) 100% of the irradiated worms regain the power to regenerate. Another

new finding was an abscopal effect, i.e., an influence of the irradiated part on the regenerative ability of non-irradiated tissue.

Combined studies of electrical and motor activity of human colon in in vitro and in vivo have demonstrated that colon muscle differs from that of small bowel in the type of motor and electrical activity, the origin and propagation of electrical activity and the electrical gradient. In contrast to small intestine, motor and electrical activity of the colon muscle are sporadic. Frequency of slow waves was highest in areas with highest resting pressure. Two pacemaker gradients exist in the colon. A decreasing gradient (similar to that of small bowel) is present in the proximal colon and an increasing gradient from transverse colon to anus. These findings may be explained on the grounds that the proximal colon as well as the small intestine originate embryologically from midgut, whereas the transverse and distal colon originate from hindgut. Furthermore, both small intestine and proximal colon derive innervation from nerves coursing along superior mesenteric routes whereas the more distal colon is innervated via inferior mesenteric routes. There is also a marked difference in function between proximal and distal colon.

These studies emphasize the need to carefully compare segments from similar areas when deriving age related information in different subjects, or longitudinally in the same subject. They also provide basic correlates between physiology and function which may be used to clarify altered colonic function with aging.

A clinical trial of the effectiveness of calcitonin administration in the treatment of osteoporosis is underway.

A previously unrecognized site of thyroid hormone binding in dog liver and kidney has been identified. The analogy of other hormone receptor proteins would suggest that these proteins in the dog or rat represent a step in at least one action of thyroid hormone. Actions of thyroid hormone(s) are known at cell membranes (heart), at cell nuclei (liver: direction of protein synthesis), and binding of T4 is known at mitochondrial and endoplasmic reticulum sites. While it is possible that proteins currently under investigation relate to cytosol transport (cell membrane to nucleus), it is clear that reversible binding of hormone by these proteins also could control availability of hormone to organelle binding sites. It is also possible that these moieties are involved in deiodination of T4 (? activation), but elaboration of radioactive iodide has not been inapparent under the conditions of our system and T3 (deiodinated T4) is also bound reversibly by the proteins. The significance of these studies, then, relates to basic mechanisms of hormone action and hormone metabolism in liver and kidney.

A new blood lipid-lowering agent, halofenate, has been found which produces profound increases in serum free T4. These studies on the mechanism of this observation have been published (J. Clin. Endocrinol. Metab., 34: 200, 1972).

Thirteen subjects have been tested for short-term memory using the signal detection procedure. Although this procedure is time consuming, it permits a separate analysis of registration and decay parameters. Data

from 8 young subjects (age 16-19) compared with 5 old subjects (age 60-75) are compared. Young subjects show a larger registration parameter and, paradoxically, there is a suggestion that they also have larger decay rates. The data are interpreted within the framework of Wickelgrin's item-time decay parameter for short-term memory. It is suggested that the older subjects shift their strategy in memory to reduce the time spent on input of a new item to memory and thereby reduce the decay of items in memory. Completion of this study will require about 15 young and 15 old subjects. If the data warrants, subjects will then be brought back for testing at different presentation rates in order to determine the decay rates during item input vs. the decay rates after an item has entered short-term memory.

Intramural Research

The Laboratory of Molecular Aging has made significant progress in developing scientific data and theory basic to the biology of aging. Research efforts, ranging from the examinations of molecular interactions, in vitro, to investigations of the integration and coordination of physiological systems in the intact animal, have focused on control and regulatory mechanisms in adult development and aging.

A chemical model has been developed and evaluated to test the hypothesis that senescence results from errors in the incorporation of amino acids into proteins caused by the mispairing of nucleotide bases during replication, transcription, or translation. It has been found that in the absence of divalent metal ions, poly(I) reacts with only the complementary cytidines in the copolymer (U,C). In the presence of the metals (MgII, MnII, NiII), the poly(I) reacts with both cytidines and uridines. Thus, divalent metal ions do induce mispairing, presumably because they stabilize polynucleotide strand interactions. Without the divalent metals these interactions are weakened and therefore are sensitive to base complementarity.

Progress has continued on the development of a technique for the sequencing of DNA, which contains the genetically determined information for growth, development, and aging. It has been found that the presence of metal ions greatly enhances the specificity of DNAase I, and that different metals produce different specificities. This effect is due to the property of metals to select one of the bases for preferential binding; the bond adjacent to the selected base is not recognized as a proper site for enzymatic attack. A method has been devised for determining whether DNAase cleaves bonds in one chain of the double helix, or whether bonds in both chains are cleaved simultaneously. Results with Cu-DNA show a lack of complementarity, suggesting that the former, single-hit, mechanism prevails.

A technique, dependent on the hybridization of poly (A) to radioactive poly (U), has been successfully developed in order to identify and quantitate message RNA in the presence of bulk RNA. This technique has been applied to show that (1) mRNA levels in blowflies drop by a factor of 10 half way through the adult flies' lifespan; and (2) RNA-containing viruses which cause cancer can be distinguished from those which do not cause cancer by the amount and length of the poly (A) sequences. Sequences in DNA have been found that are complementary to the poly (A) in RNA. These sequences

appear to be present in the double stranded form in chromosomes. New RNA-DNA hybridization conditions have been established wherein virtually all of the 28s ribosomal RNA is converted to a hybrid structure and is largely resistant to ribonuclease. A procedure for carrying out RNA-DNA hybridizations with RNA covalently linked to the filters has been successfully worked out. This system has potential application to the isolation of genes in a pure form and in determining changes during senescence.

The antiviral defense mechanisms of human diploid (WI-38) tissue culture cells have been found to change with age. Interferon is induced by poly (I) poly (C) most effectively in the middle of the in vitro lifespan and its induction falls off sharply before the cells die.

Studies on the mechanism of active transport in renal tubules have advanced significantly and several basic principles have been established. The rate of binding of D-glucose to the isolated luminal (brush border) membrane is dependent on the sugar concentration. Two saturable, 1-2 μM (Site I), and 50 mM (Site II), systems are found. The high affinity sites can bind 1.4 pmoles/mg protein and the Kglucose at Site I is 3×10^4 greater than at Site II. Binding of D-glucose, in contrast to the complete transport mechanism, does not require Na^+ and is not inhibited by ouabain. The importance of sulfhydryl groups for transport are indicated by the inhibition of binding by the diuretic, Mersalyl, and N-ethyl maleimide. Phlorizin inhibits competitively at Site I (Ki = 0.2 mM) but non-competitively at Site II. The bindings of sugars exhibit sterospecificity. L-glucose is bound at a rate 20% that of D-glucose, but by a different mechanism. Bindings of the two isomers are mutually independent; additive; L-glucose does not reverse D-glucose binding; and phlorizin does not inhibit L-glucose binding. D-galactose binding is biphasic, indicating high and low affinity sites and having, respectively, 50 and 25% the rates of D-glucose. Common binding sites for D-glucose and D-galactose are indicated by mutual competitive inhibitions. Two binding sites for phlorizin have been found, with Km values of 8 μM and 2.5 mM. Only binding at the high affinity site is Na^+ dependent. Phlorizin binding is inhibited in a competitive fashion by D-glucose. When glucose binding takes place in a Na^+-free system there is no inhibition by phlorizin in the high affinity range, although glucose binding is inhibited by the same concentration of phlorizin in the presence of Na^+. These results are consistent with the concept that phlorizin and glucose are bound at sites near to one another, to account for the mutual competitive types of inhibition, but are bound to separate sites, one Na^+ dependent, and the other Na^+ independent.

Progress has been made in defining the control mechanisms in cerebral metabolism. Hexokinase in brain is associated with mitochondria, in contrast to its location in the cytosol in most other tissues. In brain the enzyme can be reversibly bound and debound, ATP and ADP being effective solubilizers. It has been found that nucleotide debinding is dependent on the ATP:ADP ratio. At a constant total nucleotide concentration, added exogenously, solubilization increased linearly with increasing ratios. At a ratio of 4.8 mM ATP:0.8 mM ADP, the intracellular concentrations in rat brain, 4X as much enzyme was solubilized than at the reciprocal ratio. During oxidative phosphorylation, when ADP is converted to ATP endogenously, the mitochondrial hexokinase becomes solubilized. An 85% increase in the

soluble form of the enzyme is found when the physiological concentration of ATP is obtained. This change in compartmentation of hexokinase during coupled respiration, plus the finding that the soluble form of the enzyme is more sensitive to inhibition by glucose-6-P, suggest that the binding and debinding of the enzyme to mitochondria may be a factor in the physiological control of hexokinase activity, and thus the glycolytic rate, in brain.

The hypothesis of age-related alterations in the action of endocrines in regulating intermediary metabolism has been tested. The specific activities of the mitochondrial α-glycerolphosphate dehydrogenase from livers of adult (12-13 months old) and senescent (27-29 months old) hypothyroid rats have been measured at different intervals following the induction of enzyme synthesis by triiodo-L-thyronine (T_3). There are no significant differences between the two age groups in the rates of induction, the maximal levels of the induced enzyme, or the decreases in activity following the attainment of peak activities. These findings suggest that the biochemical machinery for protein synthesis in this tissue in aged animals is in no way impaired, at least in this specific model system, and that the livers of senescent rats are fully capable of responding to direct adaptive stress.

Changes in the State 3 (ADP-stimulated) and State 4 (controlled) rates of succinate oxidation in liver mitochondria induced by thyroid hormone has also been examined in adult and senescent rats. Adult animals made hypothyroid by feeding thiouracil have significantly decreased State 3 rates of respiration and respiratory control ratios. The State 4 rate of oxidation is not lowered significantly. A single injection of T_3 to these hypothyroid rats results in increases in States 3 and 4 rates as well as in the respiratory control ratios. The enhancement is evident at 6 hours after hormonal administration and reaches a maximum in about 2 days. No differences in the rates of induction nor in the maximal levels achieved are found between mature and aged animals. These findings also indicate that the livers of senescent rats respond fully to direct stimulation by thyroid hormone.

During the past year the Laboratory of Cellular and Comparative Physiology focused on studies to define the relationship between lysosomal activities and aging of cells.

Age-related changes in the activities of multiple forms of acid phosphatase in the rotifer have been estimated qualitatively. Polyacrylamide gel electrophoresis indicates the presence of 8 isozymes. Of these, 5 remain constant throughout the lifespan, whereas one decreases and two increase in activity. The activity of the isozyme which undergoes the largest change concomitant with aging becomes most marked at the cessation of reproduction, and continues to increase throughout the post-reproductive period, coincident with the onset of high mortality rates. These observations are consistent with the hypothesis that aging in the rotifer is programmed rather than stochastic in pattern.

Lysosomal function in the left ventricle of 6, 12, and 24 month old male rats has been monitored by electron cytochemical studies of acid phosphatase, β-glucuronidase, aryl sulfatase, and cathepsin B activities. These histochemical studies suggest that (1) the mechanism for lysosomal formation

is the same in all age groups; (2) the distribution of lysosomes in the tissue as well as their number do not vary with age; (3) the amount of mitochondria subject to degradation by lysosomes seems to be constant with age; and (4) the mechanisms of mitochondrial degradation by lysosomes do not differ with the age of the rat.

Ultrastructural studies of the papillary muscle in the left ventricle of the rat indicate a marked increase in the number of residual bodies as a function of age. The changes are evident by the twelfth month of age and they are significantly abundant at 24 months. Lysosomal destruction of entire papillary myocardial cells is indicated. The extent of this degradation may suggest drastic alteration in valvular function with age.

Clinical Physiology Branch. There have been two significant advances in techniques of longitudinal data analysis. One is the creation of a statistical table which will enable an investigator who is contemplating the initiation of a longitudinal study to set the duration of the study, the frequency of acquisition of data points, and the number of subjects required in order to define rates of change in selected functions with specified accuracy. This can be done if a reasonable estimate can be made of the anticipated variance and the anticipated rates of change in the function. These "reasonable estimates" will be available for many physiological, biochemical, and psychological variables from the Baltimore Longitudinal Study.

Longitudinal analyses have been carried out for two estimates of pulmonary function, the vital capacity (VC) and maximum breathing capacity (MBC). While both of these functions are known to be markedly reduced in older people, the rates of decline along the age span are quite different in these two functions. Thus a simple cross-sectional analysis of VC and MBC in adults suggests that decline in both functions is quite linear throughout adult life. The longitudinal changes that can be observed in individuals as they age, however, show that on the average MBC does not begin to decline in our participants until the 6th decade and loss of function progressively accelerates after that age. VC also shows an accelerating decline in function as age increases; here the decline is already seen in the 4th decade but the total decline in this function over the adult age span is less severe than for the MBC. The striking discrepancy between cross-sectional and longitudinal results for pulmonary function suggests that there are cohort differences between men with different years of birth which give a misleading picture of the pattern of pulmonary function decline with age. Changes in function in the early adult years are less than had been expected from cross-sectional results and in the advanced years are greater than expected. These average changes must now be subjected to further analysis to detect the influence of other variables (activity levels, smoking, etc.) on differences in rates of decline in function in individuals within each age group.

Similar to the age trends in pulmonary function were the results of analyses of renal function as determined by 24 hour creatinine clearance. Cross-sectional results suggest that decline in clearance is quite linear over the adult age span, decreasing by 9 ml per minute per decade of life. Longitudinal analysis of individual slopes, however, showed an accelerating loss of function as age advances. Young adults show a decline of only 2 ml

per minute per decade while aged adults lose 18 ml per minute per decade. The underestimate of the true rate of decline in the aged from cross-sectional analysis may be due to selectively higher mortality rates in subjects with relatively poor clearance. This possibility will be tested as our mortality experience increases.

Differences in skin temperature between right and left supraorbital areas have been measured in 495 participants in the Longitudinal Study. These subjects have been carefully assessed for clinical evidence of cerebrovascular disease. The technique has been proposed as a potential simple screening device for population groups. In our hands, however, it appears to be of very limited value since only 23% of the subjects with known cerebrovascular disease had significant temperature differences while 13% of the presumably normal controls also had positive results. It is possible that the risk of future development of clinical cerebrovascular disease will be very high in the 13% who must at this point be classified as "false positives". The longitudinal nature of the study will provide this answer in time.

Nutritional deficiencies in the aged are known to be common and are generally attributed more to the socio-economic deprivation of this group than to biological or physiological aging effects. The volunteers in the Longitudinal Study Group under investigation at the Gerontology Research Center are not a deprived group--it may be characterized as upper-middle class and has a very high educational level. It, therefore, offers a unique opportunity to study nutritional status under very favorable conditions. Abnormalities of nutritional status would be expected to be minimal in this group. We have completed the first stage of a far-reaching analysis of nutritional factors in this highly favored group in a collaborative study with nutritionists at the Philadelphia General Hospital and at the Hoffman-LaRoche Company. The status of three vitamins of the B complex were assessed and a surprising prevalence of deficiencies was discovered. Of the 200 men studied, 30% were spontaneously taking vitamin supplements to their diet--they had received no instructions from us in this regard. There was chemical evidence that 1/5 of the subjects not taking vitamins had abnormally low levels of thiamine (B_1) and riboflavin (B_2) and as many as half of the older subjects were deficient in pyridoxine (B_6). Not only were vitamin levels low, but there was also evidence of abnormal enzyme levels in the plasma and in the red blood cells. These results were in contrast to the group taking vitamin supplements. Chemical evidence of B vitamin deficiency was rare in these subjects.

A comprehensive analysis of variables influencing the determination of basal metabolic rate (BMR) has been completed. Because of the recognized variability in this measurement, we have measured three separate time periods on each of two separate days of hospitalization. It was anticipated that there would be adaptation to the procedure (the wearing of a mask, the presence of complex equipment in the environment, lying quietly in bed) so that the most basal conditions, i.e., the lowest oxygen consumption, would occur perhaps on the last run of the second day. The opposite proved true. The first value obtained gave significantly lower values than later determinations. It is now possible to reduce the multiple analyses to a single value which best characterizes each individual for use in future analyses of

rate of change in BMR with advancing age and of relations between changes in oxygen consumption and rates of aging of other functions.

The glucose-clamp technique for servo-control of the blood glucose concentration in man continues to provide interesting new insights into factors controlling insulin secretion and insulin metabolic effects. With the aid of the SAAM Computer Program we have now been able to define with confidence the size of three components of the insulin pool and the rate constants describing the exchange of insulin among these pools. Pool 1 is dimensionally the size of the plasma volume, 4.6% of body weight. Pool 2 is small, 1.9% of BW and in rapid equilibrium with Pool 1. Pool 3 is relatively large, 9.3% BW, and is in slow equilibrium with Pool 1. The total size of the three pools is essentially that of the extracellular space. We can now derive the pattern of insulin concentration changes in Pools 2 and 3 when the insulin concentration in Pool 1 only can be directly measured. The importance of this advance is shown by the fact that we now have gained an understanding of the delay in the response of peripheral tissues to changes in plasma insulin concentration. The delay is apparently determined by time needed for insulin to diffuse from Pool 1 to Pool 3, which we now believe represents the interstitial fluid of the peripheral tissues. Of direct pertinance to certain aging theories is the fact that there was no demonstrable difference in pool sizes and insulin kinetics between 16 young adult and 6 aged subjects. Thus differences in carbohydrate metabolism between old and young subjects cannot be ascribed to hinderances in diffusion of insulin across the capillary barrier or through the interstitial spaces.

An interesting new clinical syndrome of potential importance, especially to older patients, has been defined and its pathogenesis is being delineated. Water intolerance was found to be induced by cyclophosphamide (CY) administration. Hyponatremia occurred in 9 consecutive patients (8 with neoplasm, 1 with drug-induced aplastic anemia) treated with high dose CY (30-100 mg/kg/day). In these patients a syndrome was observed which consists of: (1) striking rise in urine osmolality (Uosm) despite fall in serum sodium (9 mEq/L) and plasma osmolality (17 mOsm/L) 6-12 hours after CY; (2) decreased urine output and weight gain; (3) close temporal relationship between the excretion of CY alkylating activity and changes in Uosm. Creatinine clearance remained normal. RBC volume and albumin space were unchanged after CY. All patients had normal water load tests before CY and received identical water and solute intake during CY. Water tolerance remained normal in control patients who received high dose cytosine arabinoside (50 mg/kg/day) instead of CY. The close correlation between Uosm and alkylating activity suggests that the active metabolite of CY is involved in the mechanism of water intolerance. The relationship of the CY effect to ADH release or action is unclear but tumor release of ADH seems unlikely since the syndrome occurred in one patient without demonstrable neoplasm.

The rate of metabolism of thyroxine (T_4) slows progressively with age. Studies have continued on the factors which control T_4 disposal, specifically hormonal transport and binding. It has now been found that so-called thyroxine binding prealbumin (TBPA) is a carrier for triiodothyronine (T_3) as well. The binding sites for these hormones differ, however. Much more is now known also concerning the intracellular binding protein for T_4 and T_3.

The kinetic properties of the protein(s) in kidney, small intestine, liver, and ovary are being defined. These studies are essential to an understanding of the mechanisms underlying the alterations in thyroid metabolism with age and may be analogous to studies of "receptor" proteins for the steroid hormones.

The effect of age on intrinsic myocardial function and on integrative reflex mechanisms regulating the cardiovascular system has been studied by means of "pharmacologic dissection" in young and old rats. Animals were prepared in advance by appropriate chronic implantation of catheters in the vascular system. Thus heart rate, blood pressure, cardiac output, and derived indices such as stroke work have been measured in unanesthetized and unrestrained animals. Under resting conditions cardiac performance is decreased with age as judged by lower cardiac output and left ventricular work in association with higher heart rate. Vagal tone is also lower at rest since cholinergic blockage (atropine) increases the heart rate more in young than in old animals. The older animal also shows less responsivity to direct stimulation (phenylephrine bolus injection) and a diminished cardioinhibitory baroreceptor reflex (tested by chronic phenylephrine infusion to achieve equal BP elevation in young and old animals). These results in the rat are of especial interest since they demonstrate aging effects on control mechanisms in the circulatory system which are independent of coronary artery disease. They may thus be taken as effects of aging per se.

Work with the renin-angiotensin system has involved various aspects of the chemistry of angiotensin peptides and comparative studies of analytical techniques for the measurement of renin in plasma: double isotope assay for angiotensin I (a technique developed in this laboratory), bioassay, and immunoassay. A major effort has been related to the synthesis and evaluation of a new labeled polymeric substrate for renin. The method is extremely well-suited for the study of renin inhibitors, and is currently in use for this purpose. The existence of a renin-like enzyme in plasma has been newly described using the labeled substrate. Its concentration correlates highly with that of renin. This finding suggests that the pseudorenin activity of plasma is of renal origin.

Laboratory of Behavioral Sciences. There are almost no reports in the scientific literature of experimental learning tasks which show reliable age deficits among animals. Research in this laboratory during the past year has shown that these previous failures were the result of testing animals on tasks which were too simple and that age-related learning deficits increase as task complexity increases. These findings should provide researchers with useful experimental models to test aging hypotheses in lower animals.

Intensive studies with a 14 unit T-maze--a highly complex task for rats to learn--have shown that some old animals are incapable of learning this task although all young animals can learn it. The reason old rats fail to learn the maze is that they make perseverative errors, i.e., an old rat tends to make an incorrect response consistently at the same point in the maze. Furthermore, the point in the maze where such perseverative errors occur varies from rat to rat indicating that while the behavioral mechanism

is the same from one animal to another, the effect is not due to any unique properties of the maze itself. Animals which make such perseverative errors can be taught the maze by concentrating their training into a short span of time. What is especially intriguing is that such training consistently leads to poorer learning among younger animals. These data suggest that the mechanisms of learning may be different for the elderly than they are for the young.

Senescent mice (24 month old) which are allowed to exercise voluntarily will increase their exploratory behavior during the immediate post exercise period, and for as long as 30 days after the exercise. A similar effect was observed in 24-month-old rats, and the effect of exercise was still present after two months. These findings suggest that a significant improvement in activity among old subjects is possible.

An in-line, digital computer has been added to the resources of the primate research laboratory. The computer can control multiple, simultaneous experiments in the laboratory as well as catalog data from these studies. It is anticipated that the application of this computer will expand the research activities of the section substantially. In addition to this technical achievement, there have been some noteworthy experimental findings. These will not be described here (although they do appear in the individual progress report) because the experiments need to be replicated before they are reported generally.

Longitudinal analyses of tests of serial learning and of associative learning have shown that learning ability under pacing conditions declines with age. As subjects age they make increasingly more errors when the material to be learned is presented at a relatively high rate. Detailed analyses of the data show that learning ability does not decline with age under slow pacing, except among subjects in their 70s. This finding indicates that the age-related decline in performance among the older men can not be attributed to learning ability, per se, but rather to the deleterious effects of time pressure. Further analyses of the errors made by the old men under time pressure show that the errors these men made were errors of omission. The men were unable to respond correctly and, therefore, tended not to respond at all. Errors of commission were not different among the age groups; in fact, under some conditions these errors decreased longitudinally among all age groups except the oldest (subjects in their 70s).

Studies of the effect of task complexity and learning have shown that performance declines with age. Subjects were confronted with a task which required them to learn three lettered words in which the letters were misarranged. After the subjects had learned the words, they were required to reconstitute the original misarranged word according to various sets of rules. It was observed that as the rules became less specific (e.g., "rearrange the letters to make the original word") the older subjects made more errors than the younger subjects. When the rules were quite specific (e.g., "reverse the first two letters"), the old subjects were as good as the young men. The mechanisms which underlie the coding and organization of newly acquired information seem to be fundamental to the age-related changes in learning. Further studies of information processing should help to better understand

these mechanisms.

Research on the learned control of cardiac arrhythmias has continued this year. One especially noteworthy finding is that a patient with Wolff-Parkinson-White syndrome (a disorder involving a defect in the conduction of impulses within the atrial and ventricular conduction systems) could learn to control her cardiac function. Not only could this patient learn to control her heart rate, but she also could learn to control the prevalence of her abnormal beats. She could alternately (every two minutes) produce and inhibit the rate of occurrence of her arrhythmia. This finding in the laboratory is striking since it shows that rather exquisite extrinsic neural control of the heart is possible and learnable. The clinical import of this study must await long-term follow-up studies in this patient and others.

NICHD ANNUAL REPORT
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Gerontology Research Center
Laboratory of Behavioral Sciences

There have been a number of notable scientific developments within the laboratory during the past year. Some of these results come from experimental observations, and their implications are easily documented. However, some of the developments are technical and the implications of these results only can be guessed.

There are almost no reports in the scientific literature of experimental learning tasks which show reliable age deficits among animals. Research in this laboratory during the past year has shown that these previous failures were the result of testing animals on tasks which were too simple and that age related learning deficits increase as task complexity increases. These findings should provide researchers with useful experimental models to test aging hypotheses in lower animals.

Intensive studies with a 14 unit T-maze--a highly complex task for rats to learn--have shown that some old animals are incapable of learning this task although all young animals can learn it. Experiments completed this year have shown that the reason old rats fail to learn the maze is that they make perseverative errors: i.e., an old rat tends to make an incorrect response consistently at the same point in the maze. Furthermore, the point in the maze where such perseverative errors are made varies from rat to rat indicating that while the behavioral mechanism is the same from one animal to another, the effect is not due to any unique properties of the maze itself. Additional experiments have shown clearly that animals which make such perseverative errors can be taught the maze by concentrating their training into a short span of time. What is especially intriguing about this highly reliable finding is that such training consistently leads to poorer learning among younger animals. These data suggest that the mechanisms of learning may be different for the elderly then they are for the young.

Other research in this laboratory has shown that 24 mo. old mice which are allowed to exercise voluntarily will increase their exploratory behavior during the immediate post exercise period, and for as long as 30 days after the exercise. A similar effect was observed in 24 mo. old rats, and the effect of exercise was still present after two mos. These findings suggest that significant improvement in activity among old subjects is possible.

The primate research laboratory has made a major technical advance this year. An in-line, digital computer has been added to the resources of that unit. The computer can control multiple, simultaneous experiments in the laboratory as well as catalog data from these studies. It is anticipated that the application of this computer will expand the research activities of the section substantially. In addition to this technical achievement, there have been some noteworthy experimental findings. These will not be described here (although they do appear in the individual progress report) because the experiments need to be replicated before they are reported generally.

There have been several findings from the human research programs which are noteworthy. Longitudinal analyses of tests of serial learning and of associative learning have shown that learning ability under pacing conditions declines with age: as subjects age they make increasingly more errors when the material to be learned is presented at a relatively high rate. Detailed analyses of the data show that learning ability does not decline with age under slow pacing, except among subjects in their 70's; this finding indicates that the age-related decline in performance among the older men can not be attributed to learning ability, per se, but rather to the deleterious effects of time pressure. Further analyses of the errors made by the older men under time pressure show that the errors these men made were errors of omission: The men were unable to respond correctly, and therefore, tended not to respond at all. Errors of commission were not different among the age groups; in fact, under some conditions these errors decreased longitudinally among all age groups except the oldest (subjects in their 70s).

Studies of the effect of task complexity and learning have shown that performance declines with age. Subjects were confronted with a task which required them to learn three lettered words in which the letters were misarranged. After the subjects had learned the words, they were required to reconstitute the original misarranged word according to various sets of rules. It was observed that as the rules became less specific (e.g., "rearrange the letters to make the original word") the older subjects made more errors than the younger subjects. When the rules were quite specific (e.g., "reverse the first two letters"), the old subjects were as good as the young men. The mechanisms which underlie the coding and organization of newly acquired information seem to be fundamental to the age-related changes in learning. Further studies of information processing should help to better understand these mechanisms.

Research on the learned control of cardiac arrhythmias has continued this year. One especially noteworthy finding is that a patient with Wolff-Parkinson-White Syndrome (a disorder involving a defect in the conduction of impulses within the atrial and ventricular conduction systems) could learn to control her cardiac function. Not only could this patient learn to control her heart rate, but she also could learn to control the prevelence of her abnormal beats: She could alternately (every two minutes) produce and inhibit the rate of occurrence of her arrhythmia. This finding in the laboratory is striking since it shows that rather exquisite extrinsic neural control of the heart is possible and learnable. The clinical import of this study must await long-term follow-up studies in this patient and others.

Serial No. HD-AG16 (8)(c)
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Physiological Psychology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Modification of Cardiac Arrhythmias through Voluntary Control.

Previous Serial Number: Same.

Principal Investigator: Eugene R. Bleecker, M. D. (90%)

Other Investigator: Bernard T. Engel, Ph. D.

Cooperating Units: Baltimore City Hospitals
The Johns Hopkins Hospital
North Charles General Hospital

Man Years:

Total:	1.70
Professional:	1.45
Other:	.25

Project Description:

Objectives:
1. To investigate the modification of cardiac arrhythmias through voluntary control.
2. To determine whether clinically significant therapeutic improvement can be achieved in patients with cardiac disease and cardiac arrhythmias by operant conditioning of cardiac function.
3. To identify some of the autonomic nervous system mechanisms which mediate learned control of cardiac function in these patients.

Methods Employed: Volunteer patients with heart disease and cardiac arrhythmias were recruited from the Baltimore City Hospitals and Sinai Hospital of Baltimore cardiac clinics. During the study patients were either hospitalized on the Gerontology Research Center research ward of Baltimore City Hospitals or were studied daily as outpatients.

These patients were trained in the laboratory to slow and speed heart rate (HR). During a typical experimental session the patient lay semi-reclined in a hospital bed. Arrayed in front of him was a vertical display of three

lights. The top light was red, the middle light was yellow and the bottom light was green. Each session was divided into two phases. Phase 1 was a 512 second baseline period during which automatic equipment monitored and recorded the frequency of his ventricular contractions. Phase 2 was a 1024 second training period during which the patient tried actively to control his HR.

If the patient was being trained to speed his heart, the green light would be turned on and would burn steadily throughout the training period. (No lights were on during the baseline period). When the patient speeded his HR above his baseline rate, the yellow light would go on and remain on as long as HR was greater than baseline rate. As soon as his HR fell below baseline rate, the yellow light would go off. Each patient was instructed to keep the yellow light on as much as possible. He knew that speeding his heart was the correct way to do this.

During the slowing training the procedure was identical to that outlined above for speeding except that the red light rather than the green light burned steadily, and that relatively slow HR now controlled the yellow light.

After the patient had been trained to slow his HR and to speed his HR, he was further tested under differential training conditions. During the training phases of these sessions the patient was required to alternately speed and slow his HR over four consecutive 256 second intervals. The onset of each interval was signalled by the red and green cue lights,and the patient knew that these lights meant that he should try to slow and speed respectively.

Variations in these training procedures were undertaken in specific patients in order to achieve the appropriate investigative and therapeutic goals. All patients had extensive electrographic monitoring with a portable Holter monitor. Drugs studies in selected patients were performed to identify the autonomic nervous system mechanisms of heart rate control.

Major Findings: A- Patients with premature ventricular contractions (PVCs)
Two patients with PVCs were studied:
L. R. is a 27 year old woman who had a medical history of Marfan's Syndrome,a prosthetic mitral valve because of severe mitral regurgitation and frequent, unifocal PVCs (8-10/min.) which were poorly controlled with drugs. Prior to training L. R. was admitted to the research ward for studies with autonomically active drugs. These studies indicated that increased sympathetic tone was important in PVC initiation and maintainance. L. R. was then trained for three weeks as an outpatient to control heart rate and PVCs in the laboratory. Although she did not learn to slow ventricular rate (VR), she did learn to consistently speed VR and she learned to differentially control VR. PVC incidence decreased during training especially when she learned to recognize PVCs. PVC incidence also decreased during nightly 10 hr ECG monitoring. L. R. eventually did not have PVC's in the laboratory nor during continuous, 10 hr. Holter monitoring on four separate occasions during six months of follow-up. L. R. states that she can stop PVCs and can also avoid those situations which initiate PVCs.

J. A., a 23 year old man, is being studied because of a documented history of a parasystolic ventricular focus. There is particular interest in the control of arrhythmias characterized by automaticity such as parasystolic ventricular contractions. However, examination of rhythm strips at the time of training revealed frequent coupled unifocal PVC's with no evidence of a parasystolic ventricular focus. He underwent initial training in ventricular rate control in which he learned to slow VR but did not learn to speed VR. During these aspects of training there was no significant modification of PVC frequency. J. A. had to return to college classes and training was not completed. He will be seen again for training in PVC control at the end of the present school term.

B- Paroxysmal arrhythmias:

One patient with paroxysmal atrial tachycardia (PAT), one patient with paroxysmal atrial fibrillation (PAF), and one patient with PAT, PAF, and paroxysmal atrial flutter were trained in the laboratory.

M. W. is a 35 year old practical nurse who has a 6 year history of documental PAT. She was able to slow heart rate (HR) in 10 of 20 training sessions and markedly increase heart rate in each of 10 fast training sessions. In an early speeding session she generated a sinus tachycardia of 130 beats/minute. During alternate training she was able to promptly change from HR speeding to HR slowing. During a three month follow-up she has experienced one episode of PAT which she states she was able to terminate by voluntary HR control.

W. B. is a 42 year old man with a history of a myocardapathy, aortic insufficiency and paroxysmal atrial fibrillation. His cardiac rhythm varied between normal sinus rhythm (NSR) with frequent premature atrial contractions and atrial fibrillation (AF). He could not be maintained in NSR with quinidine, propanalol and digoxin. He learned to consistently slow ventricular rate (VR) while in NSR and AF. He did not learn to speed VR above baseline levels. During alternate training sessions he demonstrated increased frequency of PACs during the VR slowing phases of training. He was unable to control PAF and presently 6 months after study is in permanent atrial fibrillation.

L. W. is a 24 year old college student with a 6 month history of a variety of tachyarrhythmias including PAT, PAF, PACs and paroxysmal atrial flutter. During training in VR slowing LW decreased VR by decreasing the frequency of rapid tachyarrhythmias in training sessions but her sinus rate of 50-60 was not decreased: LW was unable to speed VR. Monitoring during training revealed a tendency for a lower incidence of paroxysmal rapid arrhythmias. Training was not completed because LW returned to complete the spring semester of college and she will return for further training sessions after this school year.

C- Angina Pectoris:

Two patients with angina pectoris were recruited for training in heart rate slowing to determine whether this training can significantly affect the incidence and severity of anginal episodes. Each patient kept detailed records of the time, frequency, duration, treatment, and severity of angina for a period of three weeks prior to and after operant cardiac training. Patient E. S. (Class III angina pectoris) learned to slow heart rate

significantly and consistently during training and was able to slow HR without feedback and during treadmill exercise. Although he was able to terminate episodes of angina with voluntary HR slowing, the frequency of anginal episodes and the daily amount of nitroglycerin required by E.S. did not change significantly after training. C.H. (Class I-II angina pectoris) slowed VR in 14/20 slowing sessions. However, he was unable to learn to slow HR without feedback. There was no significant change in C.H.'s relatively low frequency of angina before and after training.

D- Abnormalities of cardiac conduction:

S. M. is a 29 year old woman with a three year history of intermittent Type A, Wolff-Parkinson-White Syndrome (WPW). During initial training she was able to learn to slow, speed and cyclically slow and speed ventricular rate. During the alternate training sessions she generated more normally conducted QRS complexes during VR speeding and increased WPW conduction during VR slowing. S. M. was then trained successfully to increase and decrease the frequency of both normal and WPW conduction. At present she is receiving training sessions to control normal and WPW conduction with and without feedback. She will also have drug studies to elucidate autonomic mechanisms in conduction and rate control.

Significance to Bio-Medical Research and the Program of the Institute: This project is designed to increase our understanding of the neural mechanisms of cardiac control, especially as these relate to our knowledge of the extent to which it is possible to effect such control voluntarily. These findings add further credence to the conclusions that responses mediated by the autonomic nervous system can be brought under voluntary control; and that it is possible that once such control is attained, it may have clinically useful applications.

Proposed Course of Project: A clinical study program has been established with Dr. Bernard Tabatznik, Chief of Cardiology, North Charles General Hospital. Dr. Tabatznik is carrying out a longitudinal study of mortality and morbidity in cardiac patients with histories of premature ventricular contractions. A selected series of these patients will be trained to control the prevelence of their ectopic beats, and these patients will be followed to determine the clinical effectiveness of the operant conditioning procedure.

Research in the laboratory will continue with selected patients with cardiac arrhythmias. Pilot studies will be initiated to learn if subjects can be taught to regulate blood pressures.

Honors and Awards: Dr. Engel participated in a continuing education program at the University of Wisconsin, School of Medicine entitled, "New Applications of Conditioning to Medical Practice" (November, 1971).

Dr. Engel lectured to the faculty and staff of the Department of Medicine at The University of Rochester, School of Medicine and Dentistry (March, 1972).

Dr. Bleecker lectured to the faculty and staff of the Department of Rehabilitation Medicine, New York University Medical Center (September, 1971).

Publications: None

Serial No. HD-AG2 (8)
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Physiological Psychology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Operant Conditioning of Heart Rate in Monkeys.

Previous Serial Number: Same.

Principal Investigator: Bernard T. Engel, Ph.D. (70%)

Other Investigator: George W. Ainslie, Jr., M.D. (90%)

Cooperating Units: Baltimore City Hospitals.

Man Years:

Total:	3.50
Professional:	2.10
Other:	1.40

Project Description:

Objectives: To train monkeys to voluntarily control their cardiac functions, and to use this preparation to identify the physiological and psychological mechanisms associated with this control.

Methods Employed: These experiments are done with rhesus monkeys (Macaca mulatta). The procedure involves several stages. First, a cannula is implanted into an external iliac artery under sterile surgical conditions. Following the surgery it is necessary to infuse continuously 1.5 U/ml/hr. heparinized saline into the catheter to keep the artery/cannula patent. This preparation permits adequate monitoring of blood pressure and pulse rate for at least six months. The monkey is chronically maintained in a primate chair which is situated inside of a sound-proofed booth.

All subjects are trained to change their heart rates with the same technique, using sessions about 65 minutes long. During the first 30 minutes of each session, the monkey stays in its sound-proof booth with the door shut, to allow its heart rate to reach a resting level. During the last 10 minutes of this period automatic equipment records its heart rate and systolic and diastolic blood pressures. After the animal's baseline heart rate has been determined, control modules are set so that heart periods longer or shorter than the average basal rate can be automatically detected.

During the final 35 minutes of each session, a cue light in the center of the door facing the monkey is on, warning is that it must keep one of two other lights on or receive a 0.45 second, 10 ma. electric shock to its tail. These two lights are on either side of the center light, and colored different from each other and from the center light. If the monkey is being trained to slow its heart rate, the amber light on the right is on whenever the interval between its last heart beat and the one before it was longer than a predetermined interval based on the baseline heart rate. Whenever the light is out, it is liable to be shocked according to a standardized schedule. If it is to be trained to speed its rate, the green light on the left is on whenever its last inter-beat interval was shorter than a predetermined interval; it is shocked on a similar schedule for failing to keep the light on. In essence then, the monkey must learn to keep the appropriate cue light burning to avoid a shock by controlling its heart rate. It must either slow or speed its heart depending upon which cue light is functional.

Once a subject has learned to speed and/or slow its heart, a stimulus that is known to change heart rate is presented so that the interaction of the training and the new stimulus can be studied. Techniques for introducing three kinds of stimuli have been developed in pilot experiments this year: (1) Cues signalling impending shock. It is known that monkeys (and many other species including man) raise their heart rates and blood pressures when presented with a stimulus that has been paired with unavoidable electric shock. This is part of the so-called "conditioned emotional response" (CER). It is held to be involuntary response based on classical (respondent) conditioning, in which an innate response to shock comes to be elicited by an arbitrarily chosen stimulus that regularly precedes it. An experiment was designed to test whether operant learning of heart-rate slowing can change the classically conditioned response. A monkey that had been trained to slow its heart rate by the procedure described above was given several trials of similar length, but with none of the lights lit and no shock to its tail. Instead, two minute periods of clicking from a loudspeaker alternated with two minute periods of silence. The frequency of the click alternated in turn between two and twenty clicks per second, so that the following pattern repeated itself every eight minutes: 2 minutes of 2/sec. clicks followed by 2 minutes of silence followed by 2 minutes of 20/sec. clicks followed by 2 minutes of silence. After it was ascertained that the monkey's heart rate did not change upon presentation of the clicks alone, a 0.5 second, 10 ma. shock to its tail was given at the end of every period of 2/sec. clicks. When the response was stable, the shock pattern was changed so that it was given after the 20/sec. clicks but not the 2/sec. clicks. Changes in heart rate and blood pressure were recorded. (2) Electrical stimulation of the limbic system. It is known that stimulation of certain brain structures, such as the septum, amygdala, posterior hypothalamus and cingulate gyrus, by means of steel macro-electrodes will produce temporary changes in heart rate and blood pressure. These are believed to be independent of learning or even of the subject's state of consciousness. An experiment was designed to test whether operant learning of heart rate can alter the response to stimulation of these structures. A bipolar electrode was developed, consisting of a stainless steel wire inside a hypodermic needle and protruding about one millimeter from the tip, both insulated except for their tips. A monkey which had previously been catheterized and trained to both speed and

slow his heart rate was anaesthetized, and the electrodes were implanted in its brain by means of a stereotaxi instrument. The use of nitrous oxide as an anaesthetic permitted cardiovascular responses to stimulation that closely resembled those of the waking state, and it was possible to lower the electrodes while stimulating and secure them in place with dental cement when a cardioactive site had been reached. The monkey was then returned to its chair and allowed to recover from the operation. It was initially tested in sessions of the usual length, but in which no cue lights were on and no shock given. Various amplitudes, durations, and frequencies of stimulation were applied through each of the bipolar electrodes until a combination was found that raised heart rate by about 20 beats/min. This stimulation was then given for 30 seconds every eight minutes in sessions where the subject was required to keep heart rate below a set level to avoid shock to the tail. (3) The effect of chronic training. One animal was trained to slow its heart rate. Following this training it was put on chronic 18 hr./day, 5 day/week training schedules during which it was required to keep its heart rate below certain rates. Initially this criterion heart rate was 150 beats/min, subsequently it was reduced to 140 beats/min, then 130 beats/min and finally, 120 beats/min. Whenever the monkey's rate exceeded the criterion rate, automatic equipment would tally the time. When this time tally reached 64 sec., the feedback schedule including shock would come on and remain on for about 17 min. (1024 sec). Thus, if the animal maintained its rate below criterion throughout a given 18 hr. period, it could avoid the shock schedule entirely.

Major Findings: (1) The monkey that had been trained to slow its heart rate and was then exposed to auditory stimuli followed by unavoidable shock developed marked, stable cardiac slowing during and only during the warning stimulus. Its mean drop in rate over the first ten sessions of this condition was 28 beats/min., or 16%. When the warning stimulus was changed, the new warning came to elicit cardiac slowing of the same magnitude as the old. The old warning stimulus, however, continued to elicit some cardiac slowing over 35 sessions (140 presentations) when it was not followed by shock. This is consistent with the incomplete extinction described in other conditioned-punishment experiments. The shock itself elicited a brief increase in heart rate, just as has been described for monkeys not subjected to prior operant learning of heart rate. Systolic and diastolic blood pressure dropped an average of 6% during the warning stimulus, which had also been seen during the operant slowing task alone. It should be emphasized that during the classical conditioning procedure, the monkey did not see the lights which were the cue for slowing rate, nor did it receive any feedback about its rate. Thus prior experience with operant training to slow heart rate may change the direction of the rate component of the classically conditioned emotional response. (2) In the brain-implanted monkey, two sites were found that caused a reliable increase in heart rate over a thirty second period of rapid intermittent stimulation. This was followed by compensatory slowing when stimulation stopped. These sites are probably in or near the hypothalamus, but since the monkey is still being tested, histological confirmation is not yet available. After 26 sessions (104 stimulations) in which one site was stimulated while the monkey was obliged to slow heart rate, there was no evidence that response to stimulation during training was different from response to stimulation during the baseline period. The

interaction of the stimulation and the speeding task is now being studied, without clear results as yet. (3) The monkey on the chronic training schedule developed highly reliable, extremely slow heart rates. Its average, consecutive 5 day heart rates were (in beats/min): 95,90,95,90,98, 82,75,78,81,74,83,81,84,81,86,83,82. These averages are based on a count of all heart beats during each 18 hr. training period: Thus, each number reported above is based upon a count of more than 200,000 beats. There was little difference between the animal's average heart rate on and off the feedback schedule, however, heart rates during feedback did tend to be somewhat lower. On several occasions the feedback schedule was never turned on indicating that the animal maintained its rate below the criterion for the full 18 hr. period. During some sessions the animal would maintain heart rates as low as 60 beats/min. for as long as 30 consecutive min.

Significance to Bio-Medical Research and the Program of the Institute: If it is replicated in other monkeys, the change in the CER through prior operant learning will be one of the clearest examples of a rarely described situation: the modification of classical or respondent conditioning by means of operant conditioning. In effect, the respondent and operant effects of same reinforcer, electric shock, have been placed in diametrical opposition to each other: The expectancy of shock in the respondent situation alone would be expected to make the subject speed its heart, but in the operant situation alone should make him show it. This phenomenon is also relevant to the etiology of so-called psychosomatic disease since the cardiac slowing response in the monkey we studied is clearly abnormal.

Proposed Course of Project: The Raytheon 704 computer acquired for the laboratory this year will soon be operational. As soon as this occurs it will become possible to carry out multiple simultaneous studies. This increase in the laboratory's capacity will enable us to explore each of the three effects described above in a detailed and systematic manner.

Honors and Awards: Dr. Engel delivered the Presidential Address to the Society for Psychophysiological Research.
Dr. Engel was appointed Associate Professor of Behavioral Biology at The Johns Hopkins University, School of Medicine.

Publications: Engel, B. T. Operant conditioning of cardiac function: A status report. Psychophysiology, (in press).

Serial No. HD-AG14 (8)(c)
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Physiological Psychology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Relationships between cardio-respiratory activity, behavior and aging.

Previous Serial Number: Same.

Principal Investigator: Bernard T. Engel, Ph.D.

Other Investigators: Reginald E. Quilter

Cooperating Units: Baltimore City Hospitals.

No work was done on this project during this fiscal year.

NICHD Annual Report
July 1, 1971 through June 30, 1972
Laboratory of Behavioral Sciences
Contract and Collaborative Research

Contract Number: NIH 70-2296
Contract Title: Twenty-five year follow-up analysis among former Army Air Force cadets.
Contractor: University of California (Los Angeles)
Money Allocated: $10,673 (supplement for FY 72)
36,251 (total for FY 71 and FY 72)

Objectives: In 1944 Dr. M. A. Wenger carried out a series of psychological and physiological tests on a group of 2111 Army Air Force cadets. In 1964 he sent health questionnaires to 1032 of these men and received 725 responses. On the basis of an analysis of these results it appeared that the 1944 tests might predict the occurence of some diseases among these men.

The purpose of this contract is to enable Wenger to carry out a survey of as many of the 2111 men as he can locate to determine the value of the 1944 test battery in predicting the subsequent development of certain psychological and physiological disorders. The reason this contract was initiated through this laboratory is that if the Wenger test battery does have predictive validity we would propose to include it in the longitudinal study program of the Gerontology Research Center.

Major Findings:

1. Administration

Research on this contract was begun in February, 1971 after Wenger received BMB approval to distribute a Health questionnaire by mail. Because of the delay in approval for the distribution of the questionnaire, the contractor was given an extension from 6/30/71 to 12/31/71 and additional funds in the amount of $10,673.

Difficulty was encountered in locating a large number of the subjects in the study. This problem was traced to the fact that the post office destroyed forwarding addresses after two years. In order to permit the contractor to locate as many subjects as possible, he was granted another extension to 4/30/72 without any additional funds.

2. Summary of results to date:

A. As a result of efforts to obtain current addresses from as many subjects as possible, a number of resources were uncovered. These are documented below since they may be useful to others:

1. The Disclosure Branch in the Compliance Division of the Internal Revenue Service was extremely helpful. They will provide current addresses to authorized government personnel if they can be provided with a name and with a social security number.

2. "Ham" radio operators: There are a number of "Ham" networks. Many of these are extremely cooperative, and they will petition their memberships to aid various causes. These individuals, working from

a list of names, will review local telephone directories and will provide current addresses.

3. There are sundry veteran and military resources which are variably helpful. Among these the most helpful were the VA Claim Office (VACO) and the Air Force Register.

4. The license division of the State Department of Motor Vehicles: This agency ordinarily charges a fee for this service, however, only one of the 36 states contacted refused to waive charges.

B. Through these various agencies it was ultimately possible to locate 1625 of the 2111 men in the original sample. Of these 1625 men, 180 were dead and the other 1445 returned their questionnaires. Thus the final yield was 74.8 percent.

C. Because of a death within his family, Wenger was granted another two month extension (without funds) to 6/30/72. This delay is primarily for him to draft a final report to NICHD. Since it will not be possible to include that report in this annual report, the final scientific review will be given in next year's annual report.

Significance to Bio-Medical Research and Program of the Institute: The importance of this survey is intuitively obvious. The sample of about 2000 men on whom pre-test data exist is unique. If it can be shown that these pretests can predict the emergence of subsequent diseases, then these tests will be important additions to the Longitudinal Study Project of the Gerontology Research Center. It seems highly likely that if a single test battery can predict disease after a 25 year lacuna, repeated testing such as would be possible within the Longitudinal Study Project would increase the predictive value of the tests even more. If it is possible to identify persons who are likely to develop diseases at later stages of life, it should also be possible to develop and institute preventive procedures early in life. And increasing the productive, healthy portion of the human life span is the primary goal of the Gerontology Research Center.

Proposed Course: As soon as the final report is received from the contractor, his data will be reviewed. If additional analyses are needed these will be requested. Depending upon the nature of the results of this study, appropriate experiments will be initiated within the Baltimore Longitudinal Study Project.

Serial No. HD-LB 1
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Physiological Psychology Section
4. Baltimore, Maryland

PHS-NIH

Individual Project Report

July 1, 1971 through June 30, 1972

Project Title: Behavioral Genetics and Aging.

Previous Serial Number: None.

Principal Investigator: Charles L. Goodrick, Ph.D. (30%)

Other Investigators: None.

Cooperating Units: Baltimore City Hospitals.

Man Years:

Total:	.60
Professional:	.60
Others:	.00

Project Description:

Objectives: The principal objectives of this project are (1) to determine group differences for behavioral traits and longevity among inbred strains of mice; (2) to determine : (a) heritability (degree of genetic determination vs. degree of environmental determination); (b) mode of inheritance (e.g. over-dominant, dominant, intermediate, or recessive); and (c) number of segregating units (gene blocks) controlling a particular trait (e.g. longevity); and (3) to examine relative behavioral differences among mouse strains as aging progresses.

Other objectives include analyses of the influence of diet (e.g. casein) on behavioral traits, growth, and longevity; and determination of single-gene influences upon behavioral traits, growth, and longevity.

Methods Employed: Inbred mice (C57BL/6J and A/J) of a high degree of homozygosity are maintained under uniform environmental conditions. The animals are tested behaviorally during one period of their life span, e.g., when mature, mature-old, or senile. Senility is determined as the 50% mortality point for groups maintained throughout their life span. Statistically reliable techniques have been developed to determine behaviors

relevant to natural selection such as exploration, general activity level, emotionality, simple or complex problem solving ability and taste preference. The use of segregating groups allows an estimate of the mode of inheritance, e.g., dominant or intermediate, and the number of gene blocks or segregating units controlling behavioral traits or life span. The diets used for studies in which protein intake is varied among groups of inbred and hybrid mice is isocaloric synthetic casein obtained from General Biochemicals, Chagrin Falls, Ohio. Deprived animals receive 4% casein in their diets whereas the control group receives 26% casein in its diet. Numerous kinds of mutant mice are maintained on the C57BL/6J background at the Jackson Memorial Laboratories, Bar Harbor, Maine. So far, our work has concentrated mainly on the albino mutant at the c-locus, Linkage Group 5, but we are now working with beige, yellow, and obese mutations also.

Major Findings: A. An experiment has been completed to determine the effects of aging and genetic constitution upon behavior and longevity for A/J and C57BL/6J inbred mice. A/J mice are albino (white coat), short lived behaviorally inactive mice, while C57BL/6J mice are melanic (black coat), long lived, behaviorally active mice. Both groups die of nonspecific causes. Groups of A/J, C57BL/6J, F_1 and F_2 mice have been tested on a number of behavioral measures with an N at least 16 per group at 5, 14, and 23 months of age, and at the time of advanced old age for the four groups: A/J (23 mo.) C57BL/6J (26 mo.), F_1 and F_2 (29 mo.) at the approximate time of senility or 50% mortality. Behavioral data were collected using standard tests developed in our laboratory to determine: (1) sensory discrimination, (2) preferenced for ethyl alcohol, (3) performance and retention of a learned operant response, (4) exploration behavior, and (5) emotionality. These data are in the process of analysis. B. The present experiments were designed to examine open field test differences between albino and pigmented mice tested under the conditions of bright white illumination and dim red illumination. The first experiment was to determine (a) open field responses of inbred parental and F_1 groups during a series of forced exploration tests under the condition of red or white illumination and (b) the effect of initial testing with white illumination upon scores obtained under the red illumination condition. A second experiment utilized F_2 groups of albino, black, or brown mice, while a third experiment used mutant albino mice and pigmented controls as subjects. For the first experiment the subjects were 96 experimentally naive five month-old male mice of the A/J and C57BL/6J inbred strains, and F_1 hybrids (A/J female XC57 male). The following results were obtained: For all trials mode of inheritance of exploratory activity was intermediate, while mode of inheritance of emotionality was dominant. When tested with red illumination all groups had higher scores of exploration and lower scores of emotionality, compared with scores obtained with white illumination. Albino F_2 groups and mutant albino groups did not differ from pigmented groups in total activity or emotionality. However, inbred albino and mutant albino mice did not reduce activity within trials as much as pigmented groups. Heritability estimates were consistently high over a six trial series for both exploration and emotionality, and the estimates of number of genetic factors on trials three to six were one for both scores. The conclusions of these experiments were the following: In general, albino mice are not inactive

or emotional because of some factor or factors associated with albinism. A/J albino and C57BL/6J pigmented mice represent mouse strains which differ for a remarkably high number of behavioral traits, and they may represent optimal parental strains for studies of behavior genetics if a degree of generality exists for the mode of inheritance of inbred mouse behavioral traits. The ecological significance of these data mainly concern the intermediate inheritance of exploration behavior. Traits which have dominant modes of inheritance have been suggested as subject to natural selection, while traits intermediate in modes of inheritance were neutral, without adaptive significance. However, exploration behavior clearly has adaptive significance and is of intermediate inheritance in the present study. Much more information regarding modes of inheritance of a wide spectrum of behavioral traits is required, but there is no reason now to assume that normal mode of inheritance of behavioral traits differs from mode of inheritance of physiological traits. The finding of single factors (or gene blocks) responsible for exploration (after trials 1 and 2) and emotionality may indicate that behavioral traits differ from physiological traits chiefly in their susceptibility to environmental influence. The present research forms a foundation for research now in progress concerning the interaction of heredity, age, training and environment for behavioral traits of important ecological significance. C. An experiment was initiated to examine the generality of mode of inheritance for the behavioral traits of exploration behavior, emotionality and alcohol preference using mature-young mice of four inbred strains. The inbred strains were A/J, BALB/cJ, C57BL/6J, and DBA/2J, while the hybrid groups were AxB, AxC, AxD, BxC, BxD, and CxD, and both males and females were tested for each of the ten groups (total N=360). This experiment was necessary because one of the major drawbacks to our studies of aging and genetics using A/J and C57BL/6J mice was the possible lack of generality for any results obtained. The results of this experiment are not complete. Diallel genetic analyses of variance are in progress. However, it seems clear from preliminary inspection and analyses that for these four diverse inbred strains, modes of inheritance of exploration behavior, emotionality and alcohol preference are highly consistent across strains: Exploration behavior is intermediate in mode of inheritance, emotionality is dominant in mode of inheritance, while alcohol preference is intermediate in mode inheritance. Therefore, our conclusions are that it is possible to generalize regarding mode of inheritance when just two inbred parental strains are utilized in a genetic experiment. Our analyses will be presented in greater detail in the next annual report.

Significance to Bio-Medical Research and Program for the Institute:

The study of the genetics of behavior and longevity allows an assessment of: (1) the mode of inheritance for the factor studied (i.e., dominant, intermediate, etc.); (2) the relative importance of hereditary and environmental factors; and (3) the number of genes or gene blocks which control the factor studied. Lack of adequate dietary protein is a condition which affects a large proportion of the world population. Our program attempts to determine the effect of diet (such as different proportions of protein in the total diet), during particular stages of the life span upon behavior and longevity for animal populations which differ in genetic constitution.

Studies of single-gene mutant animals are of importance because they allow the assessment of the importance of a specific genetic locus for physiological or behavioral factors.

Proposed Course of Project: Further inbred strains and F_1 hybrid groups are being studied to determine the generality of mode of inheritance for behavioral factors. Cross-sectional and longitudinal studies of mouse behavior will continue with mouse strains BALB/cJ and DBA/2J, in addition to the A/J and C57BL/6J mouse strains. The longevity of inbred and hybrid groups is also being determined. Experiments with low and normal protein diets should determine: (1) the relationships between growth rate and longevity, (2) the effect of low, normal, or high protein diets upon behavior at mature after access to these diets during various stages of development, and (3) the effects of a present diet of low, normal, or high protein upon subsequent behavior.

Honors and Awards: None.

Publications: Goodrick, C. Direct observation and automated scoring of the activity of active and inactive inbred mouse strains. Behavioral Research Methods and Instrumentation, 1971, 3, 255-256.

Goodrick, C. End bottle preferences of inbred mice during alcohol preference and fluid intake multiple-bottle test procedures. Psychonomic Science, In Press.

Serial No. HD-AG 27
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Physiological Psychology Section
4. Baltimore, Maryland

PHS-NIH

Individual Project Report

July 1, 1971 through June 30, 1972

Project Title: Exercise, general activity level and aging.

Previous Serial Number: Same.

Principal Investigator: Charles L. Goodrick, Ph.D. (30%)

Other Investigator: None.

Cooperating Units: Baltimore City Hospitals.

Man Years:

Total:	.60
Professional:	.60
Others:	.00

Project Description:

Objectives: The general objectives are: (1) to determine methods for increasing physical activity of lower animals, (2) to examine behavioral and longevity differences among animals which differ in physical activity level, and (3) to determine the physiological mechanisms underlying differences in activity.

Methods Employed: Wistar rats are placed in activity wheels and allowed limited to access to food. The result is an increment in physical activity (running) compared with normally fed animals or control animals with limited to access to food, but not placed in the activity wheels. Hungry animals may also be rewarded with food for running. Other studies utilize inbred, hybrid, and mutant mice or species which differ in activity level due to different genetic constitutions. (See project HD-LB 1 Behavioral genetics and aging).

Major Findings: A. Ninety, hybrid, 24 mo. old mice were paired on the basis of weight. One animal from each pair was allowed access to an activity wheel for a thirty day interval. Behavioral data were obtained for both groups (voluntary exercise and control) immediately after exercise

tests and again after an additional thirty days. The post-exercise test scores measured the amount of exploration which occurred in a novel open field environment. Additional data on longevity were also obtained. Because some mice were still alive (N=7), the data regarding longevity have not been analyzed. Scores of exploration were significantly higher for mice given voluntary exercise than for mice not allowed to exercise. The effect of voluntary exercise was to reduce the aging decrement normally found for exploration behavior. B. Another study examined the effect of voluntary exercise upon maze learning (14-unit T-maze) for aged rats. Twenty-four rats were started on voluntary exercise at the age of 24 months. The rats were reduced in weight to 75-80% of ad libitum weights, fed once daily, and maintained in activity wheels for a 95 day interval. During the last 20 days of the interval, the surviving rats (N=13) were given one test trial per day in the complex maze. At the end of training, the aged rats averaged 4.8 errors, a mean slightly higher than that obtained for aged rats not allowed voluntary exercise. The correlation between amount of exercise and errors was not statistically significant (rho=.02). Further research will determine whether additional voluntary exercise improves learning scores of aged rats. Groups of aged rats will be given extensive voluntary exercise starting at 20 months of age, with maze testing at the end of the exercise series. C. Research has been initiated to study the relations of voluntary exercise and age to body weight, basal metabolic rate (BMR), and food intake using inbred mice, hybrid mice, and inbred mouse mutations as experimental subjects. Previous research concerning exercise with rodent subjects used tasks which required the animals to forcibly exercise to avoid shock, to run on a moving treadmill, or to swim to escape drowning. Since all of these procedures are potentially harmful to the organism because they may be excessively stressful, voluntary exercise may be a better method of studying the effect of exercise per se, upon behavior and longevity. In the first experiment of this series, albino mutant mice and pigmented littermate control mice were tested on a series of 4 day trials. Body weights were comparable for the two groups, but mean food intake was greater for albino than pigmented mice. We had previously hypothesized that such differences were related to group differences in BMR. Measures of O_2 consumption confirmed the hypothesis of a higher BMR for albino than pigmented mice. Measures of voluntary exercise for five 4-day trials obtained similar mean scores for albino and pigmented groups, and during voluntary exercise food intake increased for both groups, but more for albino than pigmented mice. Body weight remained at the same level. This model system may be useful in the study of energy exchange relations for animals which differ both genetically and with respect to age. D. The maximal level of voluntary exercise for aged rats (26 mo. old) is normally very low. We are studying the effects of starting voluntary exercise earlier in the life span to determine the maximal increment in voluntary exercise that may be obtained. Rats started on voluntary exercise tests at 24 months of age maintained a much higher level of activity at 26 months of age than the control rats, tested when 26 months old. An additional group will start testing when 20 months old. Other groups will receive testing when 4 and 14 months old.

Significance to Bio-Medical Research and the Program of the Institute: One of the consistent findings of gerontological research is the decline in general activity level of old animals compared with young animals. It is important to determine whether quantity of activity (e.g. wheel activity) and/or quality of activity (e.g. increased exploration behavior or greater response variability) may be increased for old and senile animals. It is also important to examine the role of heredity with respect to voluntary exercise throughout the entire lifespan, and the effect of exercise upon behavioral decrements associated with advanced old age.

Proposed Course of the Project: Mature-young, mature-old, and aged rats will be trained to increase base activity level for food rewards to determine: (1) maximum increases which may be obtained, (2) the durability of increases (i.e., if increments in activity are maintained after training ceases), and (3) if increased activity level results in transfer to other tests or conditions. Data will be analyzed to determine: (1) correlations between general activity level and longevity and (2) longevity for exercised and nonexercised groups of project 1. Projects 2, 3, and 4 will be continued.

Honors and Awards: None

Publications: Goodrick, C. Variables affecting free exploration responses of male and female Wistar rats as a function of age. Developmental Psychology, 1971, 4, 440-446.

Serial No. HD-AG 25
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Physiological Psychology Section
4. Baltimore, Maryland

PHS-NIH

Individual Project Report

July 1, 1971 through June 30, 1972

Project Title: Operant performance, memory and aging.

Previous Serial Number: Same.

Principal Investigator: Charles L. Goodrick, Ph.D. (40%).

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	.80
Professional:	.80
Others:	.00

Project Description:

Objectives: Research on both human and animal subjects has shown that short-term memory deficits and motor performance deficits are major problems of aged individuals. An interpretation of maze learning differences between young and aged rats is that these differences are due to short-term memory deficits of the old animals. The general objectives of one phase of this project are: (1) to analyze complex maze learning of young and senescent animals; and (2) to determine variables which may act to enhance or retard maze learning ability. The objectives of the other phase of this project are: (1) to study age differences in motor performance during operant responding; and (2) to develop operant techniques which improve the retention of learned responses in old animals.

Methods Employed: Operant conditioning performance and retention studies have used 2-bar test boxes in which hungry animals are trained to press one bar to obtain a food reward while the alternate bar remains neutral. By increasing the complexity of the task (using two bars rather than one), it is possible to make a finer analysis of performance and the retention process. We are studying performance and retention as a function of reward schedule, and we are particularly interested in the partial rein-

forcement effect. The retention of partially rewarded responses is vastly greater than for responses continuously rewarded; and analysis of this phenomenon will provide information regarding the general retention process. A complex 14-unit multiple-T maze is also utilized. This maze has been shown to have a high degree of reliability and has been used in many major studies of aging and learning. Additional mazes of 6-units are being developed to study mastery of consecutive problems by young and senescent rats. These mazes will be used to analyze aging effects in short-term and long-term memory, and determine aging effects in proactive and retroactive inhibition. The study of these processes has been hampered by the lack of mazes of comparable difficulty.

Major Findings: A. There are two experiments which have reported consistent differences in learning using young and aged rats as subjects. Both of these studies used a 14-unit T-maze, a highly reliable test of complex problem solving. However, most research studies have failed to obtain age differences in learning for young and aged groups of rats. With few exceptions, these studies used tests which were relatively simple in design and of a low degree of complexity. The purpose of the present experiment was to compare tests which vary in complexity in order to determine the level of test complexity appropriate for the study of age differences in learning ability. The tests included were a straight runway, and 1-choice-, 4-choice-, and 14-choice-point mazes. The results for the straight runway, 1-choice maze, and 4-choice maze were negative: Age differences for time or error scores were not obtained between age groups (straight runway, N=18; 1-choice maze, N=32; 4-choice maze, N=40). For the complex 14-unit maze, differences between mature-young and aged groups were not statistically significant on the initial trials. However, on the last trials mature-young rats made significantly fewer errors and also had lower time scores than aged rats. Although fifteen out of sixteen mature-young rats had one or no errors on trial 20, just six out of sixteen aged rats had one or no errors on trial 20. The remaining ten aged rats made a consistent number of errors over the last five test trials. The six aged rats which reached one or no errors by trial 20 were not equivalent in performance to the mature-young group until the last trials. Perseverative errors were more frequent for aged than mature-young rats. Differences between age groups in percentage of six consecutive perseverative errors (based on choice points where six errors or more were made) were highly statistically significant (t (30)=3.86, $p<.001$). Mature-young rats made this type of repetitive response on just 16.2 percent of the possible occasions, while aged rats made this type of response on 47.1 percent of the possible occasions. The major finding of the present experiments was that age differences in learning were obtained for a task with a high degree of complexity but not for relatively simple tasks. Experiments with rodents which attempt to study age differences in the learning process or variables affecting the learning process should utilize tasks similar in complexity to the 14-unit T-maze used in the present research. B. In 1932 Clark Hull developed the goal gradient hypothesis. This concept attempted to explain how an effect can work backward to facilitate or inhibit an antecedent response. Hull hypothesized an excitatory gradient which increased with positive acceleration from the start of a maze to the goal box to account for the

change in behavior of an animal as it solved a maze. One of the more important deductions of this principle was the prediction of a backward order of error elimination. An experiment was initiated in this laboratory to determine the order of elimination of errors during learning of a 14-unit spatial T-maze utilizing two groups of rats, mature-young and aged, which were previously found to learn the maze at different rates. The subjects were thirty-two male Wistar albino rats, six and twenty-six months old at the time of testing, with sixteen rats at each age level. The rats were given preliminary training and adaptation, and then tested in the 14-unit T-maze for 20 days, with one trial per day. During each training trial the exact path of the rat was traced on mimeographed drawings of the maze design. Errors were plotted as a function of trials and choice point for 5 trial sequences. Error scores were obtained for progressive thirds of the maze for 5 trial segment based on: (A) choice points 1-5, 6-10, 11-14; (B) choice points 1-5, 6-9, 10-14; or (C) choice points 1-4, 5-9, 10-14. The choice points sequence used (A,B,or C) was randomly determined, but was the same for pairs of mature-young and aged rats. Scores were obtained for total errors for the (a) initial, (b) middle, and (c) last choice points of the maze sequence during trials 1-5, 6-10, 11-15, and 16-20. These scores were used in a repeated measures, 2x3x4 analysis of variance (age x trials x maze segment). Age differences in learning were highly statistically significant (F (1,30)=19.05,$p<.001$). Mature-young rats learned the maze more quickly than aged rats, and age differences were greater during the last trials than during the first trial (age x trials, F (3,90)=2.71, $p<.06$). Maze segments close to the goal area were learned more quickly than maze segments more distant from the goal area. The initial maze segment was the most difficult, the middle maze segment was intermediate in difficulty, while the least errors were made in the last maze segment (F (2,60)=98.13, $p<.001$). As trials progressed, there was an initial increase in error differences as a function of maze segment (segment a minus segment c), but a marked decrease on trials 16-20 resulted in a significant trials by maze segment interaction (F (6,180)=2.11, $p<.06$). However, when the percentage of total errors was plotted as a function of maze segment and trials, the proportional number of errors in the initial maze segment increased as a function of trials, while the proportional number of errors for the last maze segments decreased as a function of trials. These results indicate that age differences throughout training are consistent for each maze segment, so that differential goal gradients are not the causal factor for the age differences in learning. C. The typical effect in man of the crowding of practice trials in time is to diminish performance to a degree which depends upon the degree of massing. Many early experiments with rats also exist, most of which are congruent with this observation. An exception to this rule occurred in an experiment from this laboratory involving mature-young and aged albino rats. Aged rats which had failed to reach a criterion of learning a complex T-maze were given highly massed practice and quickly learned the maze. The present two experiments were designed to replicate and further investigate this phenomenon with appropriate controls not available in the earlier report.

The first experiment determined differences in rate of learning for groups of mature-young and aged rats tested under conditions of distributed or

moderately massed practice. The subjects were sixty-four male Wistar rats. At the time of testing 32 were six months old, and 32 were twenty-six months old. The ages were selected to represent fully grown young rats and rats of advanced old age. Testing in the 14-unit maze began, following standard adaptation procedures. One half of each age group were tested with one trial per day, while the remainder were tested with four trials per day, with thirty minutes between trials. All rats received a total of 40 trials and were rewarded in the goal area with one ml. of reward solution. Error scores were used to determine group differences in rate of learning. Mean errors for four trial blocks were scores used in a 2 x 2 x 10 repeated measures analysis of variance design (age x practice condition x trials). Mature-young rats learned the maze more quickly than aged rats for both practice conditions (F (1,60=109.37, $p<.001$). However, the second order interaction of age, practice condition, and trials was also statistically significant (F (9,540)=5.50, $p<001$.). This interaction reached a significant level because mature-young rats reduced errors more quickly under the condition of distributed practice than massed practice, while the aged rats reached a lower level of errors under the condition of massed practice than distributed practice. Moreover, significant differences as a function of practice condition occurred early in training for mature-young rats, but occurred late in training for aged rats. Although age differences are apparent on the first block of four trials, these differences were not statistically significant for trial 1 (mature-young mean=15.1; aged mean=16.5).

Twenty-four naive male Wistar rats 26 to 28 months old were subjects in a second experiment. The subjects were part of a larger group which were given 20 daily (1 per day) training trials. The rats utilized in the present experiment were classified as slow-learning rats if they failed to reach a criterion of four total errors or less on trials 17 to 20. The slow-learning rats were then matched on the basis of total errors on trials 17 to 20 and divided into three groups with eight rats in each group. All groups then received 36 additional trials in the maze; group one received 1 trial per day for 36 days, group two received 4 trials per day for 9 days, and group three received 12 trials per day for 3 days. Due to the large number of trials for group three, the between-trial interval during 4 trial sequences for groups two and three was ten minutes and the time between 4 trial sequences was 60 minutes. Mean error scores for blocks of four trials were used in a 3 x 9 analysis of variance design (practice condition x trials). Because Ss were matched on the basis of preliminary distributed practice scores, pretest means were very similar for the three groups. On series one, massed practice groups 2 and 3 increased error scores compared with rats of group 1 which continued distributed practice training (t (22)=2.80, $p<.02$). Both massed practice groups then reduced error scores at a much faster rate than the distributed practice group (F (2,21)=10.23, $p<.001$). Due to the above factors, the group x trials interaction was highly statistically significant (F (16,168)=7.43, $p<.001$). The finding of primary importance is that massed practice is a necessary condition for learning among many aged rats. The implications of these data are that learning deficits which occur for aged animals can be reduced or overcome through appropriate practice conditions, and that the training conditions which

optimize learning among young animals adversely affect performance among old animals. These effects of training procedure also should be explored in man.

Significance to Bio-Medical Research and the Program of the Institute: Learning and/or memory deficits represent a major problem among the aged human population. Major behavioral or physiological techniques to reduce performance deficits obtained for aged, lower animals have been studied in our laboratory. This project may facilitate research with higher organisms such as man by determining the optimal conditions for learning and for retention of learned responses.

Proposed Course of Project: Further studies are in progress to determine the nature of the partial reinforcement effect in relation to (a) time contingent vs. response contingent partial reinforcement and, (b) massed vs. distributed extinction trials. Other studies will examine age differences in operant performance as a function of response effortfulness. Maze studies will concentrate upon the effects of central nervous system stimulants on behavioral rigidity within the maze for old rats. We will also initiate preliminary studies of rote learning and perceptual learning of human subjects.

Honors and Awards: None.

Publications: Goodrick, C. Learning by mature-young and aged Wistar rats as a function of test complexity. Journal of Gerontology, In Press.

Serial No. HD-AG26

1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Physiological Psychology Section
4. Baltimore, Maryland

PHS-NIH

Individual Project Report

July 1, 1971 through June 30, 1972

Project Title: Solution discrimination, preference and fluid ingestion of rodents as a function of age.

Previous Serial Number: Same.

Principal Investigator: Charles L. Goodrick, Ph.D. (0%)

Other Investigators: None.

Cooperating Units: Baltimore City Hospitals

No work was done on this project in the current year.

Serial No. HD-AG6 (c)
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Section on Human Learning and Problem Solving
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Problem Solving and Age.

Previous Serial Number: Same.

Principal Investigator: David Arenberg, Ph.D. (50%)

Other Investigators: Phillip Thorne

Cooperating Units: Baltimore City Hospitals.

Man Years:

Total:	1.64
Professional:	0.50
Others:	1.14

Project Description:

Objectives: The general goals are to explore and identify reasoning processes in man, to determine in what ways these processes change with age, and to develop techniques for reducing age deficits in reasoning performance. In this project, reasoning is studied by using problem-solving procedures in which on-going solution behavior can be observed and quantified. Experiments are design to answer such questions as: (1) Is effectiveness in acquiring relevant information affected by aging? (2) Is effectiveness in synthesizing available information affected by aging? (3) What kinds of solution strategies are used and in what ways are they related to age? (4) How does imposing a memory load affect solution strategies for young and old adults?

Methods Employed: Experiment IIIa was designed to study the direct effects on age differences in concept identification of matching a sample of old men (60 to 70) with a sample of young men (17 to 21) on non-verbal intelligence.

A previous study of concept identification in this series (Experiment III) showed substantial age differences in identifying one-element concepts when the experimenter selected the positive and negative examples which

provide the information to permit the problem to be solved. In that study, although subjects were of average and low-average intelligence, age groups were not matched. Other studies of concept identification in the literature had found no age differences in number of correct identifications, but they had compared age groups which had been matched on non-verbal intelligence. In the present study, two samples of old subjects were included; one was matched to a young group on non-verbal intelligence, the other was not matched. The ten problems included were virtual replications of those in Experiment III.

Experiment VII is the most recent in a series of experiments designed to explore an unusual (but highly replicable) finding from cross-sectional analyses of the longitudinal study of concept identification (Experiment V). Under conditions of high initial information it was found in Experiment V that effectiveness in identifying disjunctive concepts was superior to effectiveness in identifying logically equivalent conjunctive concepts. Superior performance for disjunctive over conjunctive concepts is virtually unknown in the literature, but it was found for four independent age groups under the conditions of the longitudinal study. Attempts have since been made to identify variables and procedures which contributed to this unusual finding. In Experiment VII, the primary variable is what we have termed "linguistic congruence". In the longitudinal procedure, the positive designation is always "Died" which has negative linguistic properties. In the present study, designations for half the sample are also linguistically incongruent, whereas for the other half the designations are congruent with their linguistic properties; e.g., "Well" is the positive designation and "Sick" is the negative. Logically equivalent conjunctive and disjunctive concepts with high and low initial information gain are included.

Experiment VIII is a new concept identification study which departs substantially from all the others in this project. The intent is to explore subjects' preferences between information expressed conjunctively and information expressed disjunctively when the choices are equivalent in information gain, and then to determine whether such performances are maintained when they provide less information. It is possible to provide choices with equivalent (or non-equivalent) information gain by modifying the equivalence-of-information method developed in this Section. Two experiments have been designed in which each preference in a sequence is represented on two variables--conjunctive or disjunctive, and positive or negative; and the resultant vector of an entire sequence of preferences provides a preference summary. Each choice in the sequence to establish an overall preference is between two alternatives with equivalent information. Then alternatives are presented which provide less information for the choice which is consistent with the established preference. Strength and direction of preference will be related to preference maintenance under non-equivalent information and to correctness of solutions.

Major Findings: In Experiment IIIa, two samples of old men were compared with a young group on performance on one-element concept problems. The old group that was not matched with the young solved fewer problems than the young, a result which corroborates an earlier finding in this project (Experiment III). The old group that was matched with the young on

non-verbal intelligence solved as many problems as the young, similar to results reported from another laboratory in studies with age groups matched on non-verbal intelligence.

Data collection was initiated for Experiment VII, but there is some doubt that a general, high-school group is an appropriate sample; the task may be too difficult for them, and this obscures differences attributable to the independent variables (linguistic congruence, concept-type, level of initial information, and age if an appropriate old group can be found). It may be necessary to restrict the high-school sample to those in college-preparatory curricula, or to use college students as subjects.

Data collection for Experiment VIII will begin when the graduate student who helped design these studies of concept preference rejoins our staff this summer.

Significance to Bio-Medical Research and the Program of the Institute: Reasoning is among the most prized behaviors of man and among the most elusive for experimental study. In this project, methods have been and will be developed for obtaining quantifiable measures of step-by-step performance on reasoning problems. Some of these methods also provide patterns of responses which represent strategies in solving such problems.

Measures are obtained in current experiments to study changes in reasoning processes with age. These studies, in addition to identifying basic reasoning processes, should indicate how pervasive reasoning deficits are with age, whether education and cognitive activity mitigate such deficits, and what techniques could be used to minimize decline in reasoning.

Proposed Course of Project: Data collection will begin for Experiment VIII and continue for Experiment VII and all the longitudinal studies (Experiments I, IV, and V). A manuscript describing Experiment IIIa will be prepared for publication.

Honors and Awards: Dr. Arenberg was invited to participate in a joint panel discussion on experimental psychology and aging at the meeting of the American Psychological Association, September, 1971.

Dr. Arenberg was invited to present his research in concept identification at a colloquium of the Department of Psychology, Miami University (of Ohio), May, 1972.

Dr. Arenberg was elected Member-at-Large of the Executive Council, Psychological and Social Sciences Section, Gerontological Society. He was appointed a member of the Section Program Committee and a member of the Society Committee on Executive Affairs.

Publications: Arenberg, D. Verbal learning, memory, problem solving and aging. In Task Force on Aging Report, (Eds.) C. Eisdorfer & M. P. Lawton. American Psychological Association, in press.

Serial No. HD-AG7 (c)
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Section on Human Learning and Problem Solving
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Verbal Learning and Age.

Previous Serial Number: Same.

Principal Investigator: David Arenberg, Ph.D. (50%)

Other Investigators: Walter W. Surwillo, Ph.D.

Cooperating Units: Baltimore City Hospitals
University of Louisville (Kentucky)

Man Years:

Total:	1.62
Professional:	0.50
Others:	1.12

Project Description:

Objectives: General objectives are to discover important conditions which affect age differences in acquistion and retention of verbal material in man for two purposes: (1) an understanding of the factors which enable us to modify age differences experimentally should provide valuable information about aging processes and about psychological processes of learning; and (2) an understanding of the conditions which are particularly beneficial or deleterious to learning by the old may have implications for training old workers and education for the elderly.

Early studies in the project explored the effects of variation of time periods in acquisition of verbal material. Recent and current studies were designed to evaluate the effects of other variables, particularly the beneficial effects of augmenting visual presentation of various material to be learned.

Methods Employed: Participants in Experiments I and II were from the Baltimore Longitudinal Study who ranged in age from 25 to 79. The men in Experiment XVII were longitudinal participants with a baccalaureate degree categorized into five age groups. Participants in the other experiments

in this project were recruited from local, non-profit employment services and high schools, and they were paid to participate. These paid participants were males between 17 and 21 or between 60 and 70 years of age. Almost all of the men from the employment services were (or had been) blue-collar workers and were of average or low-average intelligence.

Although many different procedures have been used in the studies described below, two general methods can be specified: (1) rote verbal learning and (2) retention of material presented only once. In the rote studies, material is presented many times and the major focus is on improvement in learning or acquisition. In all the other studies, material is presented once and the focus is on retention.

Experiments I and II are longitudinal studies of rote learning. Experiment I is a serial learning study in which twelve familiar words are presented in the same order until the criterion of one errorless trial is reached. Participants are randomly assigned to one of the pace conditions. At the fast pace, subjects must respond to each item within 3.8 sec.; at the slow pace, within 5.6 sec. The primary dependent measure is total errors.

Experiment II is a study of paired-associate learning in which each of eight familiar words is paired with two consonants. Each set of two consonants is a signal to say the word which has been paired with it. The eight items are presented (in five different orders) until the criterion of one errorless trial is reached. Participants are randomly assigned to one of the pace conditions. At the fast pace, subjects must respond to each item within 1.9 sec.; at the slow pace, within 3.7 sec. The primary dependent measure is total errors.

Experiment XVII is a study of aging and encoding and decoding aspects of memory. Ten three-letter items are presented under one of three conditions (decoding uncertainty) for each subject, and the task is to recall the three-letter items. The three letters in each item can be encoded into a word by three different rules. Each subject is instructed about one of the rules which determines a particular level of decoding uncertainty. For example, the three-letter item "UFN" can be encoded into the word "FUN" by the following three encoding rules: (1) reverse the first two letters; (2) place the vowel in the middle, or (3) rearrange the letters. These three encoding rules provide different amounts of information for decoding the word to recall the original three-letter item: (1) no decoding uncertainty--only one possible decoding according to the rule; (2) low decoding uncertainty--two decodings possible; and (3) high decoding uncertainty--five decodings possible. After the ten items are presented, subjects are required to recall the letter groups and then the words; the number of words recalled and items recalled are the dependent measures. The experiment was designed to study the effects of decoding uncertainty on the recall of the letter-items and the words for five age-groups of educated men.

Experiment XVIII is a rote paired-associate learning study with the three input conditions used in short-term and free recall studies in this series. Acquisition trials are alternated with self-paced test trials. During the

acquisition trials, subjects (1) look at the items, (2) look at the items and listen to the experimenter read them aloud, or (3) read the items aloud. Performance during identical test trials is compared for the three conditions. Unlike the other studies using these three input conditions, each subject is randomly assigned to one of the conditions and he learns only one set of letter-word pairs.

Experiment XXI is another free recall study designed to specify the source of interference discovered in Experiment XV. In that earlier study, it was found that recall of words presented at the beginning of a list is impaired by hearing or saying all the words in the list. By systematically varying within a list which words are spoken and which are only seen, it should be possible in Experiment XXI to determine whether saying interferes with recall of the spoken word or with recall of previous words in the list.

Experiment XXIII is another study of the effects of auditory augmentation on recall of visually presented geometric figures. In a previous study (Experiment XIV), it had been found that hearing descriptions of designs as they were seen improved retention, particularly for the old. In Experiment XXIII, the procedure is similar, but the designs were made to minimize the ceiling effect (near perfect performance) for many of the young subjects, and to be equivalent to permit comparison of practice effects for both age groups under both conditions.

Major Findings: In Experiments I and II, the longitudinal studies of rote verbal learning, total errors were further analyzed into errors of omission and of commission. In serial learning, preliminary analyses had shown that total errors increased only for the two oldest groups (those over 70) under both pace conditions. Some of the young groups, particularly at the fast pace, showed a decrease in total errors between two performances at least six years apart. The decreases were predominantly attributable to fewer commission errors. At both pace conditions, errors of commission decreased for all age groups except the 70 year olds.

Preliminary analyses of total errors in paired-associate learning had shown increases for all age groups at both paces. The major component in the increases in total errors was an increase in errors of omission; commission errors changed very little for any age group at either pace.

Although it is possible that the second serial list is somewhat easier than the first, and that the second paired-associate items are somewhat more difficult, the shifts in types of errors suggest changes in strategies. Perhaps omission errors increased in paired-associate learning because some subjects perceived the second task as more difficult than the first, and chose to respond less frequently. Perhaps commission errors decreased in serial learning because some subjects discovered that decreased responding (when uncertain) improved their learning.

In Experiment XVII, five age levels and three levels of decoding uncertainty were included in a short-term-memory study. When decoding uncertainty was zero, the oldest group (70-79) recalled slightly fewer words (<7.0) than the other four age groups (7.5 to 8.5), but all groups decoded with virtually

no errors, as expected. When decoding uncertainty was low, again the four youngest groups recalled about the same number of words (7.0 to 7.7), and the oldest group (70-79) recalled fewer (<6.0). The decoding uncertainty resulted in a decrease (fewer letter-items recalled the words) in letter lists of 1.6 to 2.0 items for the groups below 70 and 2.1 items for the oldest group. When decoding uncertainty was high, the sixty-year olds performed more like the seventy-year olds (rather than like the younger groups). The groups below sixty recalled about as many words as the same age groups under low uncertainty, but the increased uncertainty resulted in a decrease in letter lists recalled (about 2.4 fewer letter items than words). Both older groups (60's and 70's) recalled fewer words with consequent decreases in recall of letter lists. Although the effects are small, the data indicate age deficits for both encoding and decoding aspects of memory.

In Experiment XVIII, paired-associate learning is compared at two ages and three input conditions, i.e., "look", "look and listen", and "say". On the basis of small samples, it appears that both young and old make most errors under the "look" or visual-only condition. However, the young seem to benefit from hearing the words but not from saying them aloud, whereas the old seem to benefit from saying the words themselves but not from hearing them said by the experimenter. No interpretations are offered, however, until the effects can be shown for larger samples.

Experiment XXI is a free-recall study using mixed lists. It was designed to explore the interference effects found previously for recall of words at the beginning of a list when all words were said aloud by the subject. In a pilot study, however, both young and old subjects, when required to say only specified words, found the "say" words so compelling that few "look" words could be recalled. Evidently the effort expended in saying only selected words changed the result so substantially that the interference effect was entirely masked. Apparently it will be necessary to change the "say" condition to "listen" (the experimenter says each word as it appears) to explore the original interference effect. Responding differentially during list presentation apparently diverts attention from the "look" words and from the memory aspects of the task; but in the "listen"condition, attention problems are minimized.

Experiment XXIII is a follow-up of a previous experiment in which it was shown that memory errors in reproducing geometric designs could be substantially reduced for the old by describing salient features of a design as it was inspected. A new set of designs was tested in a pilot study in which the designs proved usable, but the instructions needed modification. The pilot data also provided a good experiential basis for devising the current scoring system. The main study is under way to explore further the effects of auditory augmentation (in the form of verbal description of key features) on visual retention of geometric designs.

Significance to Bio-Medical Research and the Program of the Institute:
Learning is more central to experimental psychology than any other behavior of higher organisms and some of the most striking and consistently reported behavioral age differences in the gerontological literature have been found

in verbal learning performance. The experiments in this project are designed to identify basic mechanisms of learning and retention and to measure changes in these functions that occur with age.

Aging, viewed as a natural experiment in which many changes occur, permits one to identify a variety of processes which may be important underlying mechanisms of learning. In addition, knowledge about experimental variables which affect age differences will be valuable in developing techniques for optimizing learning of the older person.

Proposed Course of Project: A manuscript describing Experiment XVII will be prepared for publication. Data collection will continue for Experiments I, II, XVIII, XXI, and XXIII. The relationships between changes in learning performance and changes in blood pressure (and perhaps other physiological measures) will be explored in the longitudinal study. If the audio quality can be improved for dichotic tapes made with the Raytheon 706 computer, dichotic-listening experiments will be designed to pursue such questions as: (1) Does ear-order responding reflect some basic sequential processing in auditory perception? (2) Is ear-order responding a retrieval strategy? Dichotic tapes together with a tachistoscope will also permit the study of order of response in four-channel presentations in which a different word is presented to each eye at the same time that two other words are presented, one to each ear.

Honors and Awards: Dr. Arenberg was appointed a Fellow of the Gerontological Society by the Society Council.
Dr. Arenberg was elected Member-at-Large of the Executive Committee, Division 20 of the American Psychological Association. He was appointed chairman of the Nominating Committee and member of the Program Committee.
Dr. Arenberg was invited to participate in a symposium on "Pacing effects on learning by older individuals" at the meetings of the Gerontological Society, October, 1971.

Publications: Surwillo, W.W., Arenberg, D. Gelbart, H., Gold, P. Incidence and distribution of ear-order responding in free recall of dichotic stimuli. Psychological Report, in press.

Serial No. HD-AG8 (c)
1. Gerontology Research Center
2. Laboratory of Behavioral Sciences
3. Section on Human Learning and Problem Solving
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Perceptual Retention and Age.

Previous Serial Number: Same.

Principal Investigator: David Arenberg, Ph.D. (0%)

Other Investigators: None.

Cooperating Units: Baltimore City Hospitals.

Although data collection resumed for the only experiment currently included in this project, no progress is reported at this time.

NICHD Annual Report
July 1, 1971 through June 30, 1972
Gerontology Research Center
Clinical Physiology Branch

The primary resource for studies on aging in man for the Branch has been the Longitudinal Aging Study. The development of the panel of subjects began in 1958 and increased progressively over the past 14 years. There are now 684 active participants in the study covering the entire adult span of years. The past year has been an important one for the study since there has been a concerted effort, with significant progress, in terms of developing a comprehensive analysis of the data from a longitudinal standpoint. It has been considered necessary to have at least four data points collected over a period of time before attempting to define aging trends in individuals. This year has seen 500 of our participants reach that landmark, while 300 subjects have now achieved as many as six visits here.

To advise us in planning the logistics for the next 10 years of the study a Special Advisory Group was convened in June 1971. A summary of the current status of the study was presented to them at that time. The preparation of that report and the planning in the present year for the second stage of this report has led to new statistical and computational advances of significance not only to the Baltimore Study but also to the planning of potential longitudinal studies elsewhere. The first of these is the creation of a statistical table which will enable an investigator who is contemplating the initiation of a longitudinal study to set the duration of the study, the frequency of acquisition of data points, and the number of subjects required in order to define rates of change in selected functions with specified accuracy. This can be done if a reasonable estimate can be made of the anticipated variance and the anticipated rates of change in the function. These "reasonable estimates" will be available for many physiological, biochemical, and psychological variables from the Baltimore Longitudinal Study. These should provide a degree of science to what has previously been more of an art for the planning of longitudinal studies in the future.

Another computer program has been written which will have wide application for the construction of contingency tables. It will be especially valuable for the analysis of non-continuous variables, as for example in the comparison of differences in the rates of aging of certain functions between subjects who were destined to develop coronary disease (as determined by their long-term history in our study) and subjects who on protracted follow-up have not developed evidence of the disease. The program has great versatility in allowing for the use of mean values, differences in values, slopes of change and so forth.

The comprehensive longitudinal and cross-sectional data analysis developed last year is being carried out systematically. Several specific physiological functions show interesting results to date. Longitudinal analyses have been carried out for two estimates of pulmonary function, the vital capacity (VC) and maximum breathing capacity (MBC). While both of these functions are known to be markedly reduced in older people, the rates of de-

cline along the age span are quite different in these two functions. Thus a simple cross-sectional analysis of VC and MBC in adults suggests that decline in both functions in quite linear throughout adult life. The longitudinal changes that can be observed in individuals as they age however show that on the average MBC does not begin to decline in our participants until the 6th decade and loss of function progressively accelerates after that age. VC also shows an accelerating decline in function as age increases; here the decline is already seen in the fourth decade but the total decline in this function over the adult age span is less severe than for the MBC. The striking discrepancy between cross-sectional and longitudinal results for pulmonary function suggests that there are cohort differences between men with different years of birth which give a misleading picture of the pattern of pulmonary function decline with age. Expected changes in function in the early adult years are less than had been expected from cross-sectional results and in the advanced years are greater than expected. These average changes must now be subjected to further analysis to detect the influence of other variables (activity levels, smoking, etc.) on differences in rates of decline in function in individuals within each age group.

Similar to the age trends in pulmonary function were the results of analyses of renal function as determined by 24 hour creatinine clearance. Cross-sectional results suggest that decline in clearance is quite linear over the adult age span, decreasing by 9 ml per minute per decade of life. Longitudinal analysis of individual slopes however showed an accelerating loss of function as age advances: young adults show a decline of only 2 ml per minute per decade while aged adults lose 18 ml per minute per decade. The underestimate of the true rate of decline in the aged from cross-sectional analysis may be due to selectively higher mortality rates in subjects with relatively poor clearance. This possibility will be tested as our mortality experience increases.

Differences in skin temperature between right and left supraorbital areas have been measured in 495 participants in the Longitudinal Study. These subjects have been carefully assessed for clinical evidence of cerebrovascular disease. The technique has been proposed as a potential simple screening device for population groups. In our hands however it appears to be of very limited value since only 23% of the subjects with known cerebrovascular disease had significant temperature differences while 13% of the presumably normal controls also had positive results. It is possible that the risk of future development of clinical cerebrovascular disease will be very high in the 13% who must at this point be classified as "false positives". The longitudinal nature of the study will provide this answer in time.

Nutritional deficiencies in the aged are known to be common and are generally attributed more to the socio-economic deprivation of this group than to biological or physiological aging effects. The volunteers in the Longitudinal Study Group under investigation at the Gerontology Research Center are not a deprived group - it may be characterized as upper-middle class and has a very high educational level. It, therefore, offers a unique opportunity to study nutritional status under very favorable conditions. Abnormalities of nutritional status would be expected to be minimal in this group. We have completed the first stage of an extensive analysis of nutritional factors

in this group in a collaborative study with nutritionists at the Philadelphia General Hospital and at the Hoffman-LaRoche Company. The status of three vitamins of the B complex were assessed and a surprising prevalence of deficiencies was discovered. Of the 200 men studied, 30% were spontaneously taking vitamin supplements to their diet - they had received no instructions from us in this regard. There was chemical evidence that 1/5 of the subjects not taking vitamins had abnormally low levels of thiamine (B_1) and riboflavin (B_2) and as many as half of the older subjects were deficient in pyridoxine (B_6). Not only were vitamin levels low, but there was also evidence of abnormal enzyme levels in the plasma and in the red blood cells. These results were in contrast to the group taking vitamin supplements; chemical evidence of B vitamin deficiency was rare in these subjects.

A number of correlations must now be carried out. The longitudinal continuous follow-up of these subjects will enable us to determine whether these laboratory abnormalities,disturbing as they are, are reflected in physiological or clinical abnormalities. Will the group for which we have evidence of poor vitamin status show an accelerated rate of development of age-related diseases such as arteriosclerosis, diabetes, and hypertension?

The glucose-clamp technique for servo-control of the blood glucose concentration in man continues to provide interesting new insights into factors controlling insulin secretion and insulin metabolic effects. With the aid of the SAAM Computer Program we have now been able to define with confidence the size of three components of the insulin pool and the rate constants describing the exchange of insulin among these pools. Pool 1 is dimensionally the size of the plasma volume, 4.6% of body weight. Pool 2 is small, 1.9% of BW and in rapid equilibrium with Pool 1. Pool 3 is relatively large, 9.3% BW, and is in slow equilibrium with Pool 1. The total size of the 3 pools is essentially that of the extracellular space. We can now derive the pattern of insulin concentration changes in pools 2 and 3 when the insulin concentration in pool 1 only can be measured directly. The importance of this advance is shown by the fact that we now have gained an understanding of the delay in the response of peripheral tissues to changes in plasma insulin concentration; the delay is apparently determined by time needed for insulin to diffuse from pool 1 to pool 3, which we now believe represents the interstitial fluid of the peripheral tissues. Of direct pertinance to certain aging theories is the fact that there was no demonstrable difference in pool sizes and insulin kinetics between 16 young adult and 6 aged subjects. Thus differences in carbohydrate metabolism between old and young subjects cannot be ascribed to hindrances in diffusion of insulin across the capillary barrier or through the interstitial spaces.

A comprehensive analysis of experimental variables influencing the determination of basal metabolic rate (BMR) has been completed. The BMR has long been considered one of the primary metabolic variables of interest to gerontologists since it provides in one sense a measure of active cellular mass and in another sense a measure that could be related to the rate of aging. The analysis now being conducted undoubtedly represents the largest experience ever undertaken. Because of the recognized variability in this measurement, we have measured three separate time periods on each of two separate days of hospitalization. It was anticipated that there would be adaptation to the

procedure (the wearing of a mask, the presence of complex equipment in the environment, lying quietly in bed) so that the most basal conditions, i.e. the lowest oxygen consumption, would occur perhaps on the last run of the second day. The opposite proved true; the first value obtained gave significantly lower values than later determinations. It is now possible to reduce the multiple analyses to a single value which best characterizes each individual for use in future analyses of rate of change in BMR with advancing age and of relations between changes in oxygen consumption and rates of aging of other functions.

An interesting new clinical syndrome of potential importance expecially to older patients has been defined and its pathogenesis is being deliniated. Water intolerance was found to be induced by cyclophosphamide (CY) administration. Hyponatremia occurred in 9 consecutive patients (8 with neoplasm, 1 with drug-induced aplastic anemia) treated with high dose CY (30-100 mg/kg/day). In these patients a syndrome was observed which consists of: (1) striking rise in urine osmolality (Uosm) despite fall in serum sodium (9mEq/L) and plasma osmolality (17 mOsm/L) 6-12 hours after CY; (2) decreased urine output and weight gain; (3) close temporal relationship between the excretion of CY alkylating activity and changes in Uosm. Creatinine clearance remained normal. RBC volume and albumin space were unchanged after CY. All patients had normal water load tests before CY and received identical water and solute intake during CY. Water tolerance remained normal in control patients who received high dose cytosine arabinoside (50 mg/kg/day) instead of CY. The close correlation between Uosm and alkylating activity suggests that the active metabolite of CY is involved in the mechanism of water intolerance. The relationship of the CY effect to ADH release or action is unclear but tumor release of ADH seems unlikely since the syndrome occurred in one patient without demonstrable neoplasm.

Work with the renin-angiotensin system has involved various aspects of the chemistry of angiotensin peptides and comparative studies of analytical techniques for the measurement of renin in plasma: double isotope assay for angiotensin I (a technique developed in this laboratory), bioassay, and immunoassay. A major effort has been related to the synthesis and evaluation of a new labeled polymeric substrate for renin. The method is extremely well suited for the study of renin inhibitors, and is currently in use for this purpose. The existence of a renin-like enzyme in plasma has been newly described using the labeled substrate. Its concentration correlates highly with that of renin. This finding suggests that the pseudorenin activity of plasma is of renal origin.

The rate of metabolism of thyroxine (T_4) slows progressively with age. Studies have continued on the factors which control T_4 disposal, specifically hormonal transport and binding. It has now been found that so-called thyroxine binding prealbumin (TBPA) is a carrier for triiodothyronine (T_3) as well. The binding sites for these hormones differ however. Much more is now known concerning the intracellular binding protein for T_4 and T_3. The kinetic properties of the protein(s) in kidney, small intestine, liver, and ovary are being defined. These studies are essential to an understanding of the mechanisms underlying the alterations in thyroid metabolism with age and may be analogous

to studies of "receptor" proteins for the steroid hormones.

The effect of age on intrinsic myocardial function and on integrative reflex mechanisms regulating the cardiovascular system has been studied by means of "pharmacologic dissection" in young and old rats. Animals were prepared in advance by appropriate chronic implication of catheters in the vascular system. Thus heart rate, blood pressure,cardiac output, and derived indices such as stroke work have been measured in unanaesthetized and unrestrained animals. Under resting conditions cardiac performance is decreased with age as judged by lower cardiac output and left ventricular work in association with higher heart rate. Vagal tone is also lower at rest since cholinergic blockage (atropine) increases the heart rate more in young than in old animals. The older animal also shows less responsivity to direct stimulation (phenylephrine bolus injection) and a diminished cardio-inhibitory baroreceptor reflex (tested by chronic phenylephrine infusion to achieve equal BP elevation in young and old animals). These results in the rat are of especial interest since they demonstrate aging effects on control mechanisms in the circulatory system which are independent of coronary artery disease. They may thus be taken as effects of aging per se.

Serial No. HD-AG4(c)
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Age Changes in Human Performance

Previous Serial Number: HD-AG4(c)

Principal Investigator: A. H. Norris

Other Investigator: N. W. Shock

Cooperting Units: Baltimore City Hospitals
Dr. A. T. Welford
Department of Psychology
The University of Adelaide
Adelaide, South Australia

Man Years:

Total:	1.13
Professional:	0.13
Other:	1.00

Project Description:

Objectives: This project is designed to study the effects of aging on: (a) the physiologic responses to exercise, (b) the rate of recovery of physiologic equilibrium after exercise, (c) muscular efficiency and (d) work output and fatigue. In addition, the factors responsible for limitations of performance observed in older people will be evaluated.

Methods Employed: Measured amounts of physical work are obtained in subjects of varying ages by means of a calibrated arm ergometer and quantitative mechanical analysis of limb movement. A treadmill is used to induce higher levels o work. Measurements of oxygen uptake, CO_2 elimination, pulmonary ventilation volume, heart rate, blood pressure, and electrocardiogram are made before, during and after standardized amounts of exercise. In addition, the volume of the lungs and functional capacities of the pulmonary system are evaluated. Other studies include measurements of speed of nerve conduction, reflex latency and muscle action potentials.

Major Findings: Attempts to quantify rates of change in physiological functions have been continued. Summary analyses of regression of the pulmonary function measures vital capacity (VC) and maximum breathing capacity (MBC) on age have been performed. Linear regression coefficients were

computed for each participant who had three or more measurements of these functions. Results were summarized for the following age groups: 25-34, 35-44, 45-54, 55-64, 65-74, 75-84, and 85 years and older. The average duration of observation was 7 years.

Cross-sectional estimates of decline in VC and MBC were judged to be linear over the age groups measured and were similar in magnitude to declines previously reported for these participants. Average VC declined from 5.5 l in the subjects 25-34 years old to 3.0 l in the subjects 85 years and older. Average MBC declined from 164 l/min to 73 l/min for these age groups.

Examination of rates of change in VC and MBC within age groups showed decreases for all age groups for VC while there were decreases in MBC in the 55-64, 65-74 and 75-84 year groups and increases in MBC in the other age groups. These rates of change showed decline in slope with increase in age for both VC and MBC. Rates of change in VC decreases from -0.014 l/year in the 25-34 age group to -0.080 l/year in the 85 and over age group. Rates of change in MBC decreased from +1.5 l/min/year in the 25-34 age group to -2.0 l/min/year in the 75-84 year age group. The seven participants 85 years and older increased MBC 0.39 l/min/year.

When rate of change was plotted against age for the various age groups, an increase linear relationship was found for both VC and MBC (excepting the 85 and older group for MBC). Thus, in contrast to the cross-sectional finding of a linear decrease in function over the age span observed, there was a longitudinal finding of an accelerative trend toward decline in function over the entire age span observed. The factors which cause this increasing rate of decline in function from younger to older age groups are being sought.

Significance to Bio-Medical Research and the Program of the Institute: The decline of the ability of some older people to perform their day-to-day activities and to engage in pursuits which contribute to the economic and social strength of our society represents a national loss. Identification of the physiological, medical and social correlates of high levels of physical strength and psycho-motor performance in middle and old age, as well as declines in these abilities, should lead to techniques designed to reduce the rate of decline in performance capacities with age.

Proposed Course of Project: Appraisal of participants in the longitudinal studies will be based on measurements of muscle strength in maximum power generating ability during arm exercise. Cardiovascular, ventilatory and metabolic responses to standardized arm ergometer exercise and monitored treadmill exercise will be used to classify participants into fitness categories. Measurements of lung volumes and uniformity of pulmunary ventilatio will be made to characterize the respiratory competence of the longitudinal studies participants and to evaluate the benefits of the cessation of smokin in this group.

Honors and Awards: None

Publications: None

Serial No. HD-AG3(c)
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Longitudinal Studies of Human Physiology, Biochemistry and Psychology

Previous Serial Number: Same

Principal Investigators: N. W. Shock, Director
R. Andres, Clinical Director
A. H. Norris, Coordinator (7-1-71 to 11-15-71)

Other Investigators: D. Arenberg
M. Butler
B. T. Engel
R. Fine
C. E. Martin
C. P. Scott
J. D. Tobin

Cooperating Units: Baltimore City Hospitals, Baltimore
Dr. Bernice H. Cohen
School of Hygiene and Public Health
The Johns Hopkins University, Baltimore
Dr. Gaylord Knox
Department of Radiology
Baltimore City Hospitals
Mrs. Catherine Staneck
Design Methods Section
Data Management Branch
DCRT-NIH, Bethesda
Dr. James Schlesselman and Dr. Samuel W. Greenhouse
Epidemiology and Biometry Branch
NICHD-NIH, Bethesda

Man Years:

Total:	11.09
Professional:	3.73
Other:	7.36

Project Description:

Objectives: This project is designed to: (1) secure replicate measures of physiological, pathological, biochemical and psychological variables on

longitudinal study participants at specified intervals; (2) summarize and compare the results of testing in relation to age according to cross-sectional and longitudinal formats; (3) identify factors which may be related to changes of function over time and to age at death; and to (4) determine whether the data obtained support one or another theory as to the mechanisms responsible for age-related functional decrements.

Methods Employed: The Sample: Study participants are all male volunteers recruited by subjects already members of the program. Recruits agree to return to GRC in Baltimore for 2-1/2 days of testing at 12 or 18 month intervals, depending on age, for an indeterminate period of years. At entry into the program, 87% of subjects reported at least some college, 87% were identified with professional technical or managerial occupations, 90% were presently married, 81% described themselves as financially comfortable, and of the group who returned for the fifth visit, 90% had rated their health as good or excellent on both first and fifth visits. This homogeneous aspect of the sample is advantageous for reducing the variance due to known cultural factors; caution is, however, required with respect to generalizing from the results obtained.

Testing procedures: Since inception of the study, a major objective of data collection has been to secure uniformity in testing procedures. In the area of psychological testing, standardized tests of personality, intelligence and performance are employed in addition to especially-designed procedures to assess verbal-learning and problem-solving abilities. Systematic methods have guided inquiry tinto past smoking habits, the dietary history and areas of behavioral experience. Physical examinations and medical histories are performed with check lists to insure completeness, while the variables generated by tests of kidney function, pulmonary function, neuromuscular function, cardiovascular function, and tests of vision, hearing and reaction time follow well-defined and generally accepted procedures.

Data management: In addition to the implied obligations to protect personal privacy and to submit reports of the physical examination to designated personal physicians, primary emphasis is given to the derivation of 'best measures' which are posted for purposes of keypunching and verification. These data are then transferred into the computer file, but before being utilized, are reviewed for completeness and reasonableness. A further review is performed by summarizing test results by age groups and calendar years to be certain of the consistency of statistical parameters over the course of the study. At present, a major share of the data collected before July 1971 are in the computer file and by the end of June1972, these data will have been fully reviewed and available for analysis.

Major Findings: To date, 934 males have completed one or more visits to GRC accounting for a total of over 3950 participant visits. On January 1, 1972 some 684 subjects were actively involved, 16 were on inactive status, 94 were deceased and 140 had formally withdrawn or no longer responded to inquiry.

There have been two significant advances in techniques of longitudinal data analysis. One is the creation of a statistical table which will enable an

investigator who is contemplating the initiation of a longitudinal study to set the duration of the study, the frequency of acquisition of data points, and the number of subjects required in order to define rates of change in selected functions with specified accuracy. This can be done if a reasonable estimate can be made of the anticipated variance and the anticipated rates of change in the function. These "reasonable estimates" will be available for many physiological, biochemical, and psychological variables from the Baltimore Longitudinal Study. These should provide a degree of science to what has previously been more of an art for the planning of longitudinal studies in the future.

An important advance is the recently developed program for computing contingency tables. The program allows for correlating two variables while controlling for age and accepts both quantitative and qualitative types of information. The variables employed may be means, differences, slopes or single values and because of its general character allows for different combinations of age, values and variables to be processed to obtain percentages, chi squares, contingency coefficients and maximum likelihood ratios.

Basal metabolism: An analysis of variance was carried out on 9330 tests of basal oxygen uptake conducted on 934 male subjects aged 20-96 years. Three 10-minute test periods on each of two consecutive days for each subject were used in the analysis. Values obtained during periods when nurses observed some degree of restlessness on the part of the subject were significantly higher than values obtained when the nurses conducting the tests made no comments. It is therefore appropriate to exclude such tests as not representing basal conditions. Contrary to expectations, there was no learning or adaptation to the test situation, i.e., there was no systematic decline in oxygen uptake during successive periods on the same day, or on successive days. In fact, the mean values of tests made on the first day were significantly lower than those conducted on the second day. A similar significant progressive increase was found among the 3 tests conducted on a given day.

The variance of the 6 tests done on each individual was much less than the variance among individuals, so that differences in basal oxygen consumption among individuals can be reliably determined under the conditions imposed in the longitudinal study.

When corrected for surface area, basal oxygen consumption showed a statistically significant decrement with age (1.06 cc O_2/min/M^2 per year ±0.04) when a cross-sectional analysis was applied to the data. Whether this decrement has physiological significance or not remains to be determined by relating the basal metabolism values for the individual participants to other physiological and clinical data obtained in the longitudinal study.

Renal function: Previous cross-sectional studies have revealed a linear decrement in creatinine clearance of about 10% per decade over the age span of 20-90 years. Five different replicate cross-sectional analyses of data from the longitudinal study (207 to 565 subjects per group) give essentially the same slope of the regression of creatinine clearance on age (-8.8 to -9.8 ml per min per decade of life. Thus, cross-sectional analysis indicates a gradual linear reduction of renal function over the adult age span.

In contrast when the regression of creatinine clearance on age was calculated for individual subjects who were tested repeatedly over a 10-year period (i.e., a true longitudinal analysis) the average slope was only -2.1 ml per min per decade for subjects aged 35-44 years as compared with -18.2 for subjects aged 75-84. These results show that longitudinal age changes in renal function in the early adult years are much smaller than age differences inferred from cross-sectional studies. Thus, the measurement of age changes in individuals requires a longitudinal study. The underestimate of the true rate of decline in the aged from cross-sectional analysis may be due to selectively higher mortality rates in subjects with relatively poor clearance. Evidence to support this possibility will be sought as the mortality experience in the Longitudinal Study among older subjects increases with time.

Significance to Bio-Medical Research and the Program of the Institute: A major goal of the Longitudinal Program is a deeper understanding of age-related changes in the different organ systems, and their interrelationships. In this regard, the identification of coronary artery disease serves to characterize an age-related disease in the cardiovascular system. Numerous studies elsewhere have demonstrated that heart disease occures with increased frequency in those who are hypertensive, inactive, obese or diabetic and in those who smoke cigarettes or have elevated blood lipid levels. The potential contribution of our Longitudinal Study to this important public health problem lies in the intensity of study of a number of these variables in this study.

Collection of this body of data will place us in a position to test theories of aging with the human population of the longitudinal studies. Results of comparisons between old and young individuals are presently available, but these do not permit us to evaluate theories of aging adequately. Our ability to do so will depend in part upon a comparison of the integrated medical, physiological, psychological and biochemical profiles of the individual participant with various subsequent outcomes which we may be in a position to monitor in the same individual. Outcomes such as length of life, occurrence of disease, rate of change in a functional parameter, among other things, will be sought. Some theories assume that a few cells are lost during each unit of time so that in an individual or in an individual organ system changes of a gradual nature would be found. These theories are consistent with the summation of a series of distributed minor accidents (such as radiation hits) if only a few cells are involved each time. Other theories would relate large changes in function with the occurrence of a disease episode, or major accident resulting in a substantial loss of cells. These theories are consistent with abrupt changes in functional levels which would be maintained until another major accident results in a new level. In addition, these two widely different types of theories could be found in combination with sudden as well as slow changes operating simultaneously.

Proposed Course: Data collection will be continued as in the past, except as new equipment may provide greater capacity for accurate determinations and for automating procedures. In particular, present plans call for more sophisticated measures of auditory reaction time, the addition of fundus photography, and automated assessments of pulmonary function.

Honors and Awards: None

Publications: Shock, N. W.: Where is research heading? Med. World News, __: (Spec. Issue MWN Review Series, Geriatrics 1971), 11-15, 1971.

Shock, N.W., and D.S. Rogers: What is aging? In: V. Vintu (Editor), Scinteia Almanac 1972, Scinteia, Bucharest, Romania, 1972, pp. 325-327.

Shock, N. W. and N. Solomon: Physiology of aging. Chap. 2 in: A. Davidoff, S. Winkler, and M. H. M. Lee (Editors), Dentistry for the Special Patient: The Aged, Chronically Ill, and Handicapped, W. B. Saunders Co., Philadelphia, 1972, pp. 13-24.

Serial No. HD-AG 5 (c)
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Human Performance Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Age relationships of body composition, nutrition and physical activity

Previous Serial Number: HD-AG 5 (c)

Principal Investigators: Reubin Andres
N.W. Shock
Arthur Norris (July 1, 1971-November 15, 1971)

Other Investigators: Marlene Butler

Cooperating Units: Baltimore City Hospitals

Dr. Paul Gyorgy
Philadelphia General Hospital
Department of Public Health
Philadelphia, Pennsylvania

Dr. Myron Brin
Assistant Director for Clinical Nutrition
Hoffman-LaRoche, Inc.
Nutley, New Jersey

Professor George Vose
Research Institute
Texas Women's University
Denton, Texas

Dr. Harald Schraer
Department of Biophysics
Pennsylvania State University
University Park, Pennsylvania

Man Years:

Total:	2.67
Professional:	1.17
Other:	1.50

Project Description:

Objectives: This project is designed to describe differences in body composition, nutrition, and physical activity between males of diverse age, examine these differences in relation to age, and to determine whether such differences may influence physiological and biochemical charateristics of individual subjects.

Methods Employed: Height, weight, and body circumferences of longitudinal study participants are obtained by standard anthropometric methods. Roentgenographic and anthropometric estimates of skeletal mass are combined with height, weight, and body circumferences to provide an estimate of body fat. Other estimates of fat include skinfold thickness measurements and fat thickness measurements from X-rays. Estimates of lean body mass include: (1) basal metabolic rate determinations made with standard open circuit methods, (2) twenty-four hour urinary excretion of creatinine, and (3) total body water and extracellular water determinations made from the distribution curves of injected antipyrine and sodium thiocyanate. Nutrient intakes and eating habits are estimated from interviews and self-administered questionnaires. All such measurements are repeated according to schedule in the course of each subjects participation in the longitudinal program.

In a collaborative study with nutritionists at Philadelphia General Hospital and at Hoffman-LaRoche, Inc., the status of three vitamins of the B complex is being assessed. Excretion levels of thiamine (B_1) and riboflavin (B_2) are obtained from a 24 hour urine collection. Transketolase activity and glutathione reductase activity in red blood cells are measured and serve as additional indicators for adequacy of thiamine and riboflavin respectively.

Tests for pyridoxine (B_6) status include determinations of pyridoxal phosphate in plasma and glutamic oxalic transaminase in plasma and in erythrocytes. Amino acid load tests using tryptophan and methionine are also given. One-half of the subjects receive tryptophan in an amount equal to 25 mg per kg body weight. A subsequent urine collection is analyzed for xanthurenic acid. The rest of the subjects receive methionine, 50 mg per kg body weight. The urine collected after the methionine load test will be analyzed for cystathionine and other methionine metabolites.

Major Findings: Of the first 200 men studied, approximately 30% were taking vitamin supplements containing at least one of the three B complex vitamins mentioned above. These men had received no previous instructions from us in this regard.

Of the subjects not taking vitamin supplements, almost 1/5 had abnormally low excretion levels of thiamine and riboflavin according to the ICNND (Interdepartmental Committee on Nutrition for National Defense) criteria for measuring nutritional status. Plasma pyridoxal phosphate levels of older subjects not taking vitamins were significantly lower than those of younger subjects. Abnormal enzyme levels in the plasma and in the red blood cells were also evident. In contrast, few of the subjects taking

vitamin supplements had any chemical evidence of thiamine, riboflavin, or pyridoxine deficiency.

Significance to Bio-Medical Research and the Program of the Institute: Certain changes in organ systems with advancing age and various entities of disease are thought to be affected by diet, levels of physical activity and body composition. From the repeated assessment of these factors over time, it may be possible to determine their relative contributions to longevity and the maintenance of health and vigor in later life. Difficulties associated with obtaining estimates of eating habits, activity and body composition in the past make a prospective approach necessary for the collection of reliable information.

Nutritional deficiencies in the aged are known to be common and are generally attributed more to the socio-economic deprivation of this group than to biological or physiological aging effects. The volunteers in the Longitudinal Study Group under investigation at the Gerontology Research Center are not a deprived group - it may be characterized as upper-middle class and has a very high educational level. It, therefore, offers a unique opportunity to study nutritional status under very favorable conditions. Abnormalities of nutritional status would be expected to be minimal in this group. The nutritional effects of biological aging per se may therefore be separated from what might be called "social aging".

Proposed Course of Project: Future plans are to continue the above vitamin studies until all the longitudinal subjects have been tested. Correlations between urine tests and blood tests for the same vitamin will be done. The amount of each vitamin ingested daily from vitamin supplements will be (1) considered when looking at the test results, and (2) correlated with age. Mean daily intakes of thiamine, riboflavin, and pyridoxine calculated from a seven-day food intake record will be used to better explain the laboratory test results. Follow-up of these subjects will enable us to determine whether the group with evidence of poor vitamin status will show an accelerated rate of aging or development of age-related diseases such as arteriosclerosis, diabetes, hypertension, and arthritis.

Studies of diet, physical activity, and body composition will continue to be performed according to schedule. Correlations among the several estimates of lean body mass and of obesity will be analyzed. The power of these estimates in predicting certain deleterious aging variables will provide evidence of the relative value of these measurements.

Honors and Awards: None

Publications: None

Serial No. HD-CP2(1)(c)
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Identification and characterization of cerebrovascular disease in Longitudinal Participants and correlations with other parameters of physical and psychological function.

Previous Serial Number: HD-CP2(1)(c)

Principal Investigator: C. Paul Scott

Other Investigators: Arthur H. Norris
Reubin Andres

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	0.90
Professional:	0.90
Others:	0.00

Project Description:

Objectives: (1) To establish the prevalence and incidence rates of cerebrovascular disease (CVD) among participants in the longitudinal study. (2) To evaluate and utilize objective tests of presence, classification, and degree of CVD. (3) To correlate the presence of CVD with other measures of physiological function in longitudinal participants. (4) To provide a large population of generally healthy individuals in order to validate certain instruments described below (thermometry, tonography, and the transient ischemic attack questionnaire) and help establish normal values for these tests, previously used on rather small and ill populations.

Methods Employed: Several noninvasive, nontraumatic objective measures are being utilized to identify subjects with definite or possible evidence of cerebrovascular disease (CVD): (1) Examining physicians are alerted to CVD diagnoses by inclusion of a special form; this form, the patient, and his entire record are reviewed as necessary. (2) Careful follow-up of private physician's and hospital records is made. (3) Blood pressure and carotid pulses and bruits are examined systematically. (4) Direct thermometry of the medial supraorbital area of the forehead, as described by Austin and Sajid, is carried out under controlled conditions. (5) A questionnaire to ascertain a history of transient ischemic attacks (TIA), recently developed

at the Johns Hopkins University School of Public Health, is administered by a technician and scored according to an arbitrary standardized grading system. (6) Schiotz tonography, as described by Knox, will be utilized to measure pulse pressure. Like thermometry, this method is conceived as indicating internal carotid patency. Other procedures, such as (7) radio cine-scan cerebral transit studies, will be used where indicated by results of other tests.

Major Findings: To date, eighty-four participants have been identified to have possible or definite evidence of cerebrovascular disease; 17 of these men had two or more CVD diagnoses. Their diagnoses include: cerebral infarction 28 (cerebral thrombosis 20, cerebral embolism 2, other 6); transient ischemic attack 23; chronic brain syndrome 16; cerebral atherosclerosis 15; carotid atherosclerosis 3; and migraine 18. Considering all diagnoses except migraine, their years of birth range from 1869 to 1929 (median 1896), and the age at onset of CVD ranges from 37 to 89 (average 70.6 years); those with migraine were much younger (range 10 to 67 years, average 25.1 years old). There were no appreciable differences in year of birth or age at onset between men with "possible" or "definite" diagnoses--for example, definite cerebral infarct (#20) were aged 67.0 (average) and born in 1897 (median); possible infarcts (#8) were aged 69.0 (average) and born in 1894 (median).

Four hundred ninety-five participants were tested by the protocol outlined above from January 1, 1971 through April 1, 1972; the 495 included sixty-four of the eighty-four with CVD diagnoses. The TIA questionnaire (5) was positive in 26 of these 64 (or 40.6%); and direct thermometry (4) disclosed 15 of 64 (23.4%) with temperature differences between the two median supraorbital regions greater than 0.30 degrees centigrade. This compares with 14.2% positive TIA questionnaires and 13.2% temperature differences greater than 0.30 degrees in the 431 participants tested with no CVD diagnosis.

Significance to Bio-Medical Research and the Program of the Institute: Cerebrovascular disease is a major cause of death and morbidity in aging, and means are now being devised and tested to screen normal populations for precursors of the disease. The knowledge gained with better characterization of those individuals in the longitudinal study may lead to improved preventive measures and treatment of this illness.

Proposed Course of Project: By June 30, 1972, the entire active longitudinal study population will have been surveyed by the above protocol; and that data will be entered into computerized files, to be tested for prevalence and cardiac function, as well as the effect of CVD on such psychologic and physical functions as learning and problem solving and motor tests, will be determined. Additionally, a judgement of the usefulness of the TIA questionnaire and direct thermometry test will be made.

Honors and Awards: None

Publications: None

Serial No. HD-CP4(9)(c)
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Pulmonary Function Studies: Spectral analysis of cardiopulmonary parameters as a means of quantifying cardiopulmonary function

Previous Serial Number: HD-CP4(9)(c)

Principal Investigator: Ronald Fine

Other Investigators: Phillip Thorne
Arthur H. Norris

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	0.90
Professional:	0.90
Other:	0.00

Project Description:

Objectives: The objective of this study is to characterize various indices of cardiopulmonary function by simple, noninvasive, and highly automated techniques which will permit efficient longitudinal monitoring of age-related changes. Comparisons of these methods with older more conventional ones will be accomplished, so that statements of relative accuracy and reliability can be made.

Methods Employed: Magnetic tape recordings of the physiological parameters to be analyzed will be fed through an analog-to-digital converter and digitalized data will thus be obtained. With the NIH Fortran Subroutine #SSP096 and the necessary additional software for interfacing, the frequency content of these recorded signals will be calculated. Two types of analyses will be performed: first, several mechanical properties of the Lung-Tracheobronchial System (LTBS), e.g., Dynamic Compliance (DC) and Total Pulmonary Resistance (TPR), will be determined by a Frequency Response approach, and the results compared with these same properties ascertained by conventional methods; second, phonocardiographic records will be analyzed for frequency patterns, and the regressions on age of the individual acoustic frequencies within the Heart Sounds will be studies. The Raytheon Series 700 Computer System at the Gerontology Research Center will be used for analyses.

Technique #1--Esophageal pressure is well known to be a reasonable approximation to intrapleural pressure, the driving force of the LTBS. Such pressure is easily monitored from a thin latex balloon, 10 cm long and 1-1.5 cm in diameter which has been swallowed into the mouth to lower third of the esophagus and subsequently filled with several c.c. of air. Pressure as sensed by the ballon is transmitted over thin polyethylene tubing to a Statham pressure transducer. Mouth pressure is simultaneously monitored and subtracted withing the transducer from the esophageal pressure, so that the pressure drop across the lung, or transpleural pressure, is obtained. The transducer output signal is then amplified and recorded on an oscilloscope, magnetic tape recorder, writing device, or as is needed.

The LTBS output, or airflow signal, is obtained along with the volume signal from an electronic spirometer into which the subject continuously breathes. These signals are processed in the same way as the pressure.

The signals are next digitalized and then analyzed for frequency content. Graphs are then constructed as follows: harmonic frequency is plotted as abscissa; the ratio of harmonic amplitudes of flow over pressure becomes the ordinate. This is a socalled "Bode Plot." From the shape of the Plot, the number of lumped reactive elements in the system under analysis, i.e., the number of compliances, inductances, etc. and their magnitudes, can be determined.

Technique #2--Phonocardiograms will be recorded in standard fashion and then subjected to frequency analysis. Then with a series of low pass filters of progressive lower frequencies, the same phonocardiogram recordings and analyses will be repeated so that specific frequencies can be correlated with the various components of the Heart Sounds. (This is done by noting at what filter frequency each component of the Heart Sound(s) successively disappears.) The regressions of the acoustic frequencies on age is calculated after sufficient data has been recorded.

Major Findings: An assembly language subroutine has been written which permits analog-to-digital conversion via a Call from a Fortran Main Program and returns an Array of digitalized data to the Main Program. Software for interfacing of an oscilloscope and an X-Y Plotter with the main computer has also been constructed. The Fourier Subroutine has in addition been adapted to the performance of the specific projects described herein.

Because of rapid advances in the methods used to characterize the many genetic variants of alpha-1-antitrypsin deficiency, we have postponed analysis of the lyophilized plasma samples that we have available on the subjects of the Longitudinal Study. When an appropriate analytical system is available, we will apply it to our stored sample resource.

Significance to Bio-Medical Research and the Program of the Institute: Modern advances in technology and data processing have permitted lengthy and complicated problems to be solved and large volumes of data to be analyzed in amazingly short periods of time. Because of this, the amount of useful information which can be extracted from experimental signals and systems has been greatly augmented. With appropriate algorithms and hard-

ware, mechanical properties of the lung such as DC and TPR, can be determined using entire respiratory cycles of transpleural pressure, tidal volume, and tracheal air flow, rather than sampling such curves at a few representative points and performing tedious calculations by hand as is done conventionally. The benefits of such analyses are twofold: first, a more accurate measure of the physical property sought is achieved; second, transcription and calculation errors are minimized.

Spectral analysis of Heart Sounds permits objective measurement of the frequency distribution of Cardiac Sound Energy. With such data, correlations between specific frequencies and the mechano-acoustic events which produce them can be effected (see Symposium on Cardiovascular Sound: American Journal of Cardiology, August 1971, Aldo Luisada, Ed.). Such correlations provide for an indirect, noninvasive means of following age changes in the individual cardiac structures whose vibrations produce each individual sound frequency.

Spectral analysis then is a tool with which maximal use of experimental data is achieved; also, conglomerate phenomena can be "dissected," so that simultaneous and/or nearly simultaneous events may be separately monitored. In this way subtle changes in cardiopulmonary physiology, as might occur with advancing age, and not apparent by other techniques, may be detectable.

Proposed Course: A Pilot Study with the techniques described above will first be done to verify the feasibility of the methods. If successful, the Pilot Study will be expanded into a cross-sectional age study with the Longitudinal Participants at the Gerontology Research Center as subjects. If the usefulness of these methods in Gerontology is confirmed, a longitudinal age study will be initiated.

Honors and Awards: None

Publications: None

Serial No. HD-AG1(c)7
1. Clinical Physiology Branch
2. Human Performance Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Marital, Sexual, and Social Factors in Aging

Previous Serial Number: Same

Principal Investigator: C. Martin

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	0.44
Professional:	0.44
Other:	0.00

Project Description:

Objectives: Major objectives are to: (1) describe the effects of generation and age upon marital adjustment, sexual reactivity, and sexual performance; (2) determine whether individual differences in sexual functioning may be related to some aspect of health, physiologic state or psychologic trait; and to (3) discover whether differences in marital, sexual, or social background may be associated with specific diseases or with longevity.

Methods: In the course of participation in the longitudinal program, each subject is asked to contribute an interview dealing with his history of marriage and sexual activity. The investigator, in making this request, carefully describes the objectives of the study, provides assurance of confidence and emphasizes the voluntary nature of such a contribution.

Cooperation on the part of participants has been excellent. Thus far, the results of 547 completed and 22 partially completed interviews are now entered into the computer master file. Only nine participants have declined interviews.

Participants are also requested to complete the Your Activities and Attitudes questionnaire on their first, fifth, and ninth visits to GRC. Although the questionnaire was initially designed to provide scores indicative of personal adjustment, a number of items on the questionnaire pertain to demographic, attitudinal, and behavioral variables which are utilized in other contexts.

These methods of data collection make available a range of personal and behavioral attributes by which to classify participants at the time of entry into the program, at predetermined visits during the course of observation, and according to a variety of marital, sexual, and social backgrounds.

Major Findings: New findings are unavailable at this time. Delays in analysis this past year were accounted for by the need to complete the entry of physiologic and other data in the computer master file and by the lack of a computer program for correlational analysis. Both these impediments are now largely overcome. However, the investigator was asked to become Acting Head of the Human Performance Section on November 15, 1971 and the additional duties involved have left but few hours for the present project. The time that was available was largely taken up by interviews.

Significance to Bio-Medical Research and the Program of the Institute: With respect to cited objectives, it is well to note that there are apparently no other data on sexual functioning which are both comprehensive and systematic in character and which pertain to middle-aged and older men. The fact that such data are available for individuals on whom many kinds of medical and physiologic data exist presents an unusual opportunity to relate the various measures obtained for evidence of association.

Moreover, a basic strategy employed in clinical, epidemiologic and longitudinal investigation concerns the search for factors that may be related to a given diagnosis within the experience of individuals. For the most part, what factors are considered as possible etiologic factors tend to antedate diagnosis by relatively short intervals of time. On the other hand, significant factors may antedate diagnosis by many decades. In this situation, retrospective inquiry into the early years of the marital and sexual history has the advantage of providing access to information about these early years which tends to be well-recalled. In any event, the question of whether such variables as age at first coitus, quantity of sexual activity between 20 and 40 years of age, and number of coital partners are consequential for later health is worthy of investigation.

Proposed Course: A program for computing contingency tables for data in the master file is expected to be available within weeks. This capability will be immediately put to use with respect to stated objectives.

Honors and Awards: None

Publications: None

Serial No. HD-CP 1 (c)
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Cardiovascular Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: A programmed stress test for the electrocardiographic diagnosis of coronary artery disease

Previous Serial Number: HD-CP 1 (c)

Principal Investigators: Donald A. Rothbaum
Charles S. Angell

Other Investigators: N. W. Shock
Reubin Andres

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	0.20
Professional:	0.10
Other:	0.10

Project Description:

Objectives: This test is designed to increase the sensitivity of the electrocardiographic diagnosis of latent or potential coronary artery disease in the longitudinal program participants at the CRC. It is based upon the observation that myocardial oxygen requirement increases with increasing heart rate (as an indicator of increasing cardiac output). The ability of the coronary artery system to supply oxygen to the myocardium will thus be stressed most at the maximum heart rate of any given patient. The standard double Master's two-step test does not lead to maximum or even near-maximum heart rate in most subjects and would therefore be a less sensitive test for early coronary artery disease than a test which permits graded increasing exercise to levels which can exceed the arbitrary limitations of the two-step test.

Quantitation of the differences in specificity and sensitivity of the Master's and stress exercise tests will be obtained (1) by cross-sectional correlations with other known predictors and indicators of coronary disease and (2) by longitudinal long-term follow-up of the natural history of the subjects. There have been no untoward effects of testing during the 3 years that the test has been in use.

Methods Employed: Routine resting EKG's, Master's Tests, and the Programmed Stress Test are performed and interpreted according to the World Health Organization's modification of the Minnesota Code. All participants of the Gerontology Research Center's longitudinal study in whom it is not contraindicated are studied. Limb leads and precordial leads are attached in the usual manner. The treadmill is programmed to accelerate from 0 to 3.5 MPH in the first minute of 0° grade. Subsequent increase in work load is provided by an increase in grade of 3° every minute. Subjects walk until heart rate plateau is achieved or until the test is stopped for one of the reasons previously outlined.

Major Findings: Cross-sectional analysis described by the following table documents the fact that more positive responses are obtained using the Programmed Stress Test than by using the Master's Test. Fifty-two additional subjects were added to the group during the past year.

STRESS TEST

Master's Test	Positive	Borderline Prob. Pos.	Borderline Prob. Neg.	Negative	Totals
Positive	6	0	1	0	7
Borderline Prob. Pos.	7	3	3	5	18
Borderline Prob. Neg.	8	2	39	41	89
Negative	13	3	26	334	374
Totals	34	8	69	380	488

Presumably this represents increased sensitivity in the electrocardiographic detection of occult coronary artery disease, but long-term follow up is required to rule out the possibility of decreased specificity (false positives) of the Stress Test. Too few subjects have had enough morbid events to allow conclusions about prognostic significance of a positive response at this time.

Significance to Biomedical Research and the Program of the Institute: Stress electrocardiography has been shown to be a sensitive predictor of ischemic heart disease. The opportunity to correlate data obtained using this test with data from a large number of additional known and suspected predictors of coronary heart disease, and to do so longitudinally on a stable population should greatly increase understanding of the natural history of coronary heart disease.

Proposed Course of Project: The value of the stress electrocardiogram as a predictor of overt coronary heart disease is currently being evaluated in a major review of coronary risk factors currently being undertaken.

Honors and Awards: None

Publications: None

Serial No. HD-CP6(c)
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Cardiovascular Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Non-invasive evaluation of ventricular function in man by measurement of systolic time intervals

Previous Serial Number: HD-CP6(6)

Principal Investigators: Donald A. Rothbaum
Charles S. Angell

Other Investigator: N. W. Shock

Cooperating Units: Baltimore City Hospitasl

Man Years:

Total:	.80
Professional:	.60
Others:	.20

Project Description:

Objectives: Several investigators have established the validity of measuring systolic time intervals (STI's) by non-invasive means as measures of ventricular function in man. Work from other centers has indicated that there may be age differences in some intervals suggesting a decline in ventricular function with age in man. This study is being undertaken with the purposes of: (1) confirming differences in systolic time intervals among different age groups in cross-sectional studies; (2) determining changes in STI's in individual participants in the longitudinal study; (3) seeking a mechanism for any observed age changes by pharmacologic and/or physiologic intervention, and (4) establishing a sensitive and objective clinical indicator of deteriorating ventricular function in the subjects participating in the longitudinal aging program of the Gerontology Research Center.

Methods Employed: Systolic time intervals are measured in man by simultaneous high speed recording of a single lead of the electrocardiogram, the phonocardiogram, and the externally

recorded carotid pulse, on an oscillographic recorder. Specific intervals measured include: (1) R - R interval, (2) QRS duration, (3) QS_2, the total duration of systole, from the onset of the QRS complex to the first high frequency aortic component of the second heart sound, (4) LVET, the left ventricular ejection time, measured from the onset of the upstroke of the carotid pulse to the nadir of the dicrotic notch. From these four intervals are calculated three additional parameters; (1) heart rate (reciprocal of R - R interval); (2) the pre-ejection period (PEP), PEP = QS_2 - LVET; and (3) the ratio of PEP/LVET. In addition a detailed current smoking, drug use and activity history is obtained from each subject and blood pressure is measured by cuff, immediately after each test. An estimate of average daily activity in calories is derived from a self-administered detailed activity questionnaire.

Work done by Weissler and others has demonstrated that pre-ejection period increases, LVET decreases, and the ratio PEP/LVET increases with deteriorating ventricular function as measured by independent methods at cardiac catherization.

Major Findings: Since last year data analysis on the systolic time intervals have been completed. Duplicate tracings and tracings of subjects on cardioactive drugs were eliminated, technically inadequate tracings were discarded, and approximately 100 new subjects were added to the study before final evaluation. The final group consisted of 336 participants in the longitudinal aging program of the Gerontology Research Center, ranging in age from 20-89 years. Of these 11 had heart disease with congestive heart failure, 67 had compensated heart disease, and 30 had diastolic hypertension (diastolic blood pressure $\geq$ 90 mm Hg).

Several investigators have demonstrated the negative linear correlation between heart rate and the intervals QS_2, LVET, and PEP. Our final regression equations, given below, are almost identical to our preliminary equations reported last year which demonstrates the stability of these relationships.

$$QS2 = (-2.27 \times \text{HEART RATE}) + 546$$

$$LVET = (-1.71 \times \text{HEART RATE}) + 410$$

$$PEP = (-0.56 \times \text{HEART RATE}) + 136$$

The intercepts of these three equations are referred to respectively as the QS2 index (QS2I), ejection time index (ETI), and pre-ejection period index (PEPI). These indices compare with values published by Weissler of QS2I = 546, ETI = 413, and PEPI = 131.

Blood pressure was found to have a significant effect on the systolic time intervals at levels of systolic blood pressure geater than 130mm Hg. and diastolic blood pressure levels greater than 90 mm Hg. The prolongation of ejection time with increasing systolic blood pressure might be predicted from the higher ultimate level of blood pressure necessary to eject a normal stroke volume into a less compliant arterial system. The prolongation of pre-ejection period with increasing diastolic blood pressure (afterload) would be expected because of the higher intraventricular pressure necessary to open the aortic valve.

A number of cross-sectional observations made last year have remained unaltered despite the deletion of subjects with unsatisfactory or duplicate tracings and the addition of new subjects. Systolic blood pressure increased, and activity decreased with age (both, $p < .001$). When the entire population over the age span 20-89 years was examined, no significant correlations could be demonstrated for any of the systolic time intervals or their indices with age. However, when the PEP or PEPI was plotted versus age in a scatter plot, a relationship was evident: up to age 60 PEP and PEPI both increased slightly, but highly significantly ($p < .001$) with age, at a rate of about 5 msec. decade. Beyond age 60, however, variance increased and the parameters tended to decline from their peak at age 60. The same observation of correlation of the interval with age for subjects under age 60 was found for both the QS2 and QS2I ($p < .02$ and $p < .005$ respectively). When the systolic time intervals were examined in our population with heart disease there were small increases in both PEP and PEPI and decreases in LVET and LVETI, but these were not statistically significant. A similar lack of significant variation in STI's with mild heart disease has been noted in other studies.

Significance to Bio-Medical Research and the Program at the Institute: The measurement of STI's provides a convenient non-invasive method of measuring ventricular function in a large number of individuals. The observation of increasing pre-ejection period up to age 60 with a slight decrease thereafter is most intriguing, and suggests perhaps an analogy with age changes in blood pressure in large populations. There may be two (or more) populations, one of whom has an increasing pre-ejection period with age and hence deteriorating ventricular function with an increased risk of dying young and a second population whose indices do not change with age and do not have deteriorating ventricular function.

However, our study and the studies of others have shown that the STI's are probably not sufficiently sensitive to detect subtle deterioration of cardiac function in an individual

patient due to the very small absolute changes in these measurements even with clinically obvious cardiac disease.

Proposed Course of Project: Because of the very small changes in STI measurements in clinically obvious cardiac disease and the almost comparable individual variability in the same subject upon repeat measurements, it is felt that the measurement of STI's would not be of use in predicting cardiac deterioration in individual subjects on a longitudinal basis.

Honors and Awards: None.

Publications: None

Serial No. HD-CP9
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Cardiovascular Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Myocardial oxygenation in the ischemic zone and myocardial salvage in acute myocardial infarction.

Previous Serial No:

Principal Investigators: Charles S. Angell
Kenneth A. Rosenberg
Phillip R. Thorne
Donald A. Rothbaum

Other Investigators: N. W. Shock

Cooperating Unit: Baltimore City Hospitals

Man Years:

Total:	0.4
Professional:	0.4
Others:	0.0

Project Description:

Objectives: Coronary insufficiency is conceptualized as an unfavorable balance between myocardial oxygen supply and myocardial oxygen consumption. In acute myocardial infarction, the salvageability of the ischemic zone is dependent on this relationship, and it appears that salvage requires increased oxygen delivery without a concomitant inappropriate increase in myocardial oxygen consumption. Pharmacologic maneuvers and mechanical circulatory assistance have been only partially successful. The present study is designed to investigate characteristics of myocardial oxygenation in the ischemic zone, determine its salvageability by increased coronary blood flow, and study associated changes in ventricular function and the metabolic status of the heart.

Methods: An open chest, intact canine heart preparation will be used. Intra-myocardial oxygen availability will be evaluated using a polarographic technique employing an amperometric platinum-iridium oxygen cathode 150μ in diameter.

Electrical properties of the infarcted area will be studied using intramyocardial electrocardiograms obtained from the same electrode that is used for measurement of intramyocardial oxygen availability. Ventricular function will be assessed by evaluating systemic and left ventricular pressure and dP/dt. The metabolic status of the heart will be assessed by studying coronary arterio-venous lactate-pyruvate ratio differences. Myocardial infarction will be produced by coronary artery ligation. Appropriate catheters for pressure measurement and blood sampling will be placed under fluoroscopic guidance. Control measurements of hemodynamic, metabolic, and electrocardiographic parameters will be compared to corresponding measurements under hemodynamic stress and before and after coronary artery ligation.

Major Findings: To date 7 animals have been used in preliminary trials to evaluate systems and perfect techniques. Major systems are operational at the present time. These include: polarography, cardiac catheterization, intramyocardial electrocardiography, the recording apparatus, lactate and pyruvate analysis, and the thoractomy procedure. Data collection using this preparation is scheduled to begin shortly.

Significance to Biomedical Research and The Program of the Institute: One of the most urgent problems in the coronary care unit is the management of the patient with an acute myocardial infarction who develops failure or cardiogenic shock. Many of these people have large infarcts and many have extensions of their initial infarcts into what appears to be a zone of borderline ischemic muscle which invariably surrounds the area of necrotic tissue that is the infarct. Since this ischemic zone and with it the patient's life is in such jeopardy, much attention has accordingly been given to investigation of the characteristics of potentially salvageable heart muscle. The present study is pertinent to biomedical research because evaluation of oxygen availability in potentially salvageable zones of the myocardium would bear directly on the treatment of people with acute myocardial infarction. In addition, our studies of myocardial oxygenation would be helpful in guiding work in this area by defining in conjunction with evaluation of hemodynamic, metabolic and electrocardiographic parameters exactly how much of an increase in myocardial oxygenation is required to salvage the ischemic zone. Circulatory assist devices could then be tested easily to see if they fulfill the necessary criteria. Thus, direct application of the results of these studies to patients with acute myocardial infarction may be promptly forthcoming.

Proposed Course of Poject: The preparation that will be

used in this project is flexible and allows variation of coronary blood flow, coronary perfusate, aortic pressure, heart rate, inhaled oxygen concentration and numerous additional parameters, evaluation of the effects of which would likely be quite meaningful. The project at the present time is still in its infancy; institution of the initial experimental protocol is scheduled promptly.

Honors and Awards: None

Publications: None

Serial No. HD-CP10
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Cardiovascular Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Age differences in cardiovascular performance and baroreceptor control mechanisms in intact unanesthetized rats.

Previous Serial Number: Same

Principal Investigators: Donald A. Rothbaum
Charles S. Angell

Other Investigator: N. W. Shock

Cooperating Unit: Baltimore City Hospitals

Man Years:

Total:	1.80
Professional:	1.40
Other:	0.40

Project Description:

Objectives: In order to study mechanisms of hemodynamic age changes known to occur in man, procedures must be used which are not applicable to humans. Hence it is necessary as a first step to determine whether similar age changes occur in experimental animals. This project has been undertaken, then, in an effort to 1) describe hemodynamic changes which occur with age as measured in intact, unanesthetized rats, and 2) explore age differences in cardiovascular control mechanisms.

Methods Employed: Under pentobarbital anesthesia PE 50 polyethylene catheters are placed in the left common carotid artery and both external jugular veins of 12 and 24 month old male rats, and the rats are allowed to recover. Phasic and mean arterial blood pressure are measured by means of a Statham strain gauge transducer, cardiac output is measured by an indicator dilution technique using the ^{86}RB isotopic method described by Sapirstein, and heart rate is measured from the phasic blood pressure recording. From the indices heart rate,

mean blood pressure cardiac output and body weight are derived cardiac index, stroke volume, stroke index, peripheral vascular resistance, left ventricular minute work, LV work index, stroke work and stroke work index. Animals are studied on postoperative day 9. The animals are placed in small, non-confining plexiglass cages, and lengths of PE 50 polyethylene tubing are attached to the arterial and venous catheters. In this way, the animals can be studied without further handling, and are relatively unstressed. Animals were studied under various experimental circumstances: before and after either propranolol, atropine, or a combination of the two drugs, and after hypertension induced by phenylephrine infusion.

Major Findings: Significant age differences were found between young and old animals in both the resting and hypertensive states.

1. At rest the heart rate was significantly higher and the cardiac output, cardiac index, stroke output, stroke index, and left ventricular and stroke work indices were significantly lower in the twenty-four month old animals than in the 12 month old animals.

2. Cholinergic blockade with atropine caused a significantly greater increase in heart rate in young than in old animals.

3. Beta adrenergic blockage with propranolol and a combination of beta adrenergic blockade and cholinergic blockade resulted in equivalent decreases below resting heart rate in both age groups.

4. Hemodynamic alterations induced by adrenergic nervous systems blockade did not differ between the two age groups.

5. At equal doses of phenlyephrine in bolus injection, the peak systolic blood pressure increase was greater in young than in old animals.

6. At equal systolic blood pressure increases induced by continuous phenylephrine infusion, the baroreceptor-mediated bradycardia was greater in young than in old animals.

7. The baroreceptor reflex was completely abolished in young and old animals by atropine pre-treatment but was not altered by propranolol pre-treatment.

At rest, these studies demonstrate a decrease in cardiac performance and vagal tone in the 24 month old rat. With alpha adrenergic stimulation, the control mechanisms at the peripheral arterial level and at the cardio-inhibitory baroreceptor reflex level were attenuated in the old animals.

Significance to Biomedical Research and the Program of the Institute: In a model free of arteriosclerotic heart disease, in which physiologic control mechanisms are intact, age related changes could be demonstrated at rest, in response to cholinergic blockade, and in response to acute hypertensive stress. Changes in cardiac performance due to aging may be the result of alterations in both intrinsic myocardial function and the integrative reflex mechanisms regulating the cardiovascular system.

Proposed Course of Project: This project has been completed.

Honors and Awards: None

Publications: Weisfeldt, M.L., Wright, J.R., Shreiner, D.P., Lakatta, E., and Shock, N.W.: Coronary flow and oxygen extraction in the perfused heart of senescent male rats. J. Appl. Physiol. 30: 44-49, 1971.

Weisfeldt, M.L., Loeven, W.A., and Shock, N.W.: Resting and active mechanical properties of trabeculae carneae from aged male rats. Am. J. Physiol. 220: 1921-1927, 1971.

Serial No. HD-AG 10 (c)
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Effect of age on carbohydrate metabolism

Previous Serial Number: HD-AG 10 (c)

Principal Investigators: Jordan Tobin
Reubin Andres
Karl Kramer

Other Investigators: Paul Insel
Ralph DeFronzo

Cooperating Units: Baltimore City Hospitals

Man Years:
Total: 4.10
Professional: 1.30
Other: 2.80

Project Description:

Objectives: This project is part of the long-term prospective study of human aging, the Longitudinal Study of the Gerontology Research Center. The complex interplay of aging, carbohydrate metabolism, and diabetes mellitus offers a prototype of one of the serious challenges to clinical gerontology, the differentiation of normal aging changes from specific disease states. The objectives of this project are: (1) to describe cross-sectional changes during the entire adult span of life in performance on the commonly-used clinical tests for the diagnosis of diabetes, (2) to provide age-adjusted standards of normality for these tests, (3) to define the relative sensitivity and specificity of these tests, (4) to establish the role of certain experimental variables which influence performance on these tests, (5) to investigate the mechanisms underlying the age changes, and (6) to evaluate the significance of the decline in tolerance [a] by noting the influence of poor performance on mortality and [b] by testing for correlations between performance and certain other variables known to be clearly associated with the overt diabetic state.

Methods Employed: Subjects are primarily those in the Longitudinal Study supplemented by college students and laboratory and hospital employees to increase the sample size of the 3rd and 4th decades. Subjects are given

one of the following tests per visit: oral glucose tolerance (OGTT), intravenous glucose tolerance (IVGTT), cortisone glucose tolerance (CGTT), or intravenous tolbutamide response (TRT). On the fifth visit the cycle is re-started. Diabetic patients are available primarily from the Baltimore City Hospitals Out-Patients Department; "chemical" diabetics and a small number of overt diabetics are available from the Longitudinal Study Group.

In a series of studies on the mechanism of the age change the servo-control "glucose-clamp" technique is used: control of arterial blood glucose is placed under the investigator's control. In some studies glucose-1-C^{14} is injected intravenously either as a single shot or as a continuous infusion following a prime. The course of the specific activity of circulating glucose is used to compute glucose production (primarily by liver), glucose uptake(equals production when steady-state glucose concentration is maintained), and glucose space. An Amberlite anion exchange resin is used to separate glucose in plasma from charged labeled metabolites of glucose and recycling error is corrected by established techniques.

Major Findings: We reported last year on the necessity of adapting the glucose-clamp technique so that large numbers of subjects could be studied. A method that would allow us to avoid brachial arterial catheterization was described in which superficial venous blood draining the hand was arterialized using an electrically-heated glove. The method proved to be less than ideal because of the lack of uniformity of heating. We therefore devised an insulated heated box in which the air temperature was maintained at 66-70°C. We again challenged the system by creating conditions that would be expected to cause large arteriovenous glucose differences, i.e. marked arterial hyperglycemia accompanied by hyperinsulinemia. The results show that under these conditions the A-V glucose differences were less than 1 mg% greater than they were under control conditions (basal glucose and insulin concentrations).

Furthermore there were no differences in the effect on four young adult subjects (24-32 years) and seven older subjects (55-74 years). The subjects felt comfortable with one hand heated by this technique and no systemic effects were noted.

Two assays new to this laboratory are being developed and collaboration has been established for three additional assays. We have set up the radio-immunoassay for growth hormone and the Kitabchi rat skeletal muscle protease technique for assaying proinsulin appears promising. In preparation for comprehensive studies in the coming year we have secured the collaboration of scientists at the Southwestern Medical School and the Johns Hopkins University School of Medicine for assay of glucagon, catecholamines, and adrenal corticosteroids.

Significance to Bio-Medical Research and the Program of the Institute: The "testing of tests" in clinical use for the diagnosis of diabetes is a serious one. The present study fulfills most of the recommendations for future research needs made by the Committee on Statistics of the American Diabetes Association. This report dealt only with the oral glucose toler-

ance test. The intravenous variant of this test is gaining in popularity especially among clinical research workers interested in the relation of atherosclerosis to metabolic factors. There is however no large scale study available comparing the results to these two tests. Similarly the tolbutamide and cortisone tests remain of controversial value and their present study will offer the most extensive evidence on the relative advantages and defects of these procedures.

The intercorrelations among the variables noted in the Proposed Course below will provide the most comprehensive evidence yet available on the roles of these important but poorly quantitated parameters on the development of diabetes and its complications as they are related to advancing age.

Proposed Course of Project: The longitudinal study of the diagnostic tests for diabetes is currently being analyzed by a comprehensive computer program. The cut-off date for this analysis was April 1, 1972 and, in association with other variables in the Longitudinal Study which are also now in the process of computation, will allow significant advances to be made in meeting goals (1) to (4) and goal (6). The interrelated variables of most immediate interest are the following: (a) glucose and insulin on the several clinical tests for diabetes, (b) serum lipids, cholesterol and triglyceride, (c) body composition, lean body mass and obesity, (d) physical activity levels, (e) dietary factors, and (f) presence or absence of clinical atherosclerotic disease.

Honors and Awards: Dr. Andres was an invited speaker at the Swedish Nutrition Foundation's Symposium on Nutrition in Old Age at Saltsjobaden, Sweden August 16-18, 1971.

Dr. Andres was an invited speaker at the First Rocky Mountain Symposium on Aging August 25-27, 1971, at Colorado State University.

Dr. Andres was an invited speaker at the American College of Physicians Course on Diabetes Mellitus in Indianapolis in October, 1971.

Dr. Andres was a speaker on the Maryland Medic network on the topic of Clinical Aspects of Aging.

Dr. Andres served as a resource person for the Education and Demonstration Group at the 1971 White House Conference on Aging.

Dr. Andres was appointed as member of the Medical Advisory Board of the Levindale Home for the Aged in Baltimore.

Dr. Andres was appointed as a member of a Special Committee of the Baltimore City Commission on the Aging to investigate homes for the care of the aged in Baltimore.

Dr. Andres was appointed as a member of the Education Committee of the Gerontological Society.

Publications: Andres, R.: Carbohydrate metabolism in old age. In Carlson, L.A. (Ed.): Nutrition in Old Age. Stockhold, Sweden, Swedish Nutrition Foundation, 1972 (in press).

Andres, R.: Envejecimiento y diabetes. Clinicas Medicas de Norteamerica 55: 835-846, 1971.

Andres, R: Envelhecimento e diabete. Clinica Medica da America do Norte 55: 835-846, 1971.

Andres, R., and Tobin, J.D.: Aging, carbohydrate metabolism and diabetes. In: Proceedings of the 9th International Congress of Gerontology, Kiev, (in press).

Serial No. HD-CP 7 (c)
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The kinetics of insulin metabolism in man: Relations to glucose metabolism, to age, and to obesity

Previous Serial Number: HD-CP 7 (c)

Principal Investigators: Paul Insel
Jordan Tobin
Karl Kramer
Reubin Andres

Other Investigators: Ralph DeFronzo
*Mones Berman
**Isaac Hantman

Cooperating Units: *Mathematical Research Branch, NIAMD

**Computer Systems Laboratory
Division of Computer Research & Technology

Man Years:

Total:	3.95
Professional:	2.75
Other:	1.20

Project Description:

Objectives: These studies are aimed at delineating various parameters of insulin release, distribution, and metabolism in man and the changes in such parameters that occur in altered physiologic states, including aging, diabetes mellitus, and obesity. Because of insulin's role as a prime determinant of glucose utilization, a definition of insulin kinetics will provide a more precise understanding of how the metabolism of glucose is controlled. Current studies are being performed to develop an insulin kinetic model, a glucose kinetic model, and to describe the homeostatic relationship between those two models in normal, diabetic, and obese man of differing ages. From such an analysis, important physiologic controls will be described and clues to pathogenesis of altered metabolic states may be forthcoming.

Methods Employed: The glucose clamp technique was utilized to maintain

human subjects at basal arterial blood glucose concentrations after experimental perturbations of the arterial plasma insulin level. The clamp technique employs a controlled variable-rate IV infusion of glucose solution with rapid measurement of blood glucose concentration by an automated method. A negative feed-back formula is applied at 5 minute intervals in order to servo-correct the glucose infusion rate; thus, the blood glucose concentration is maintained at euglycemic levels. The amount of infused glucose, an index of glucose utilization, provides an estimate of sensitivity to exogenous insulin.

Intravenous infusions of insulin were given by 2 techniques: (1) single-shot intravenous injection of .025 crystalline porcine insulin per kg body weight or (2) prime plus continuous infusion at 1 or 2 mU per kg body weight per minute. The prime was given over a 10 minute period such that the total insulin infused in that time was twice the rate infused continuously from 10 to 80 minutes. The infusion during the 10 minute prime was at a logarithmically falling rate which approached the continuous infusion rate asymptotically. The goal of this technique was to create a nearly instantaneous square wave of insulin concentration at a new steady state level.

In some studies glucose-1-^{14}C was injected as a primed continuous infusion during the pre-clamp period to achieve a steady state specific activity, and specific activity of glucose (corrected for metabolites and recycled glucose by established procedures) was followed after the administration of insulin. Such radioactive glucose data allows determination of endogenous glucose production and of glucose utilization as a means of analyzing insulin action.

Plasma immunoreactive insulin concentrations, plasma glucose radioactivity, and plasma glucose concentrations were utilized in mathematical modeling of insulin kinetics and glucose kinetics by the SAAM program of Berman and Weiss to determine compartmental dimensions, transfer rates, and to investigate the mechanisms by which insulin regulates glucose utilization.

Major Findings: (1) Insulin plateau concentrations, metabolic clearance rates of insulin and "post hepatic" insulin delivery rates were reported in Annual Report 1971 and revealed similar findings for young and old subjects.

(2) The SAAM program has allowed derivation of a three compartment insulin model which describes the results from all types of insulin studies. This model includes Compartment 1 (4.6 + 0.3% body weight, presumably the plasma space) in rapid equilibrium with Compartment 2 (1.9 + 0.6% body weight, presumably including interstitial fluid beds of small volume and high blood flow) and in slow equilibrium with Compartment 3 (9.3 + 0.8% body weight, presumably interstitial fluid beds of large size and low blood flow, i.e., muscle and adipose tissue). The total insulin space was 15.7 + 2.0% body weight, closely approximating independent estimates for extracellular fluid. No significant age differences were noted for exchange constants between compartments (n = 16 young adult and 6 old subjects).

(3) The time course of the glucose administration needed to maintain euglycemia, a measure of glucose utilization, did not correlate with the time course of plasma insulin concentration but closely paralleled the computed insulin concentration pattern in Compartment 3. Thus, this compartment appears to regulate the rate of glucose utilization.

(4) The SAAM program has allowed derivation of a 3 compartment glucose model which describes the results of ^{14}C glucose studies. In this model the total glucose space was 19.5 + 2.3% body weight. Preliminary analysis suggests that model-derived parameters are similar for young and old subjects and that glucose utilization can be accurately "controlled" by the linear accumulation of insulin in our compartment 3 (n = 11).

An abstract based on the findings described in item 2 have been accepted for presentation at the annual meeting of the American Diabetes Association in June, 1972 and were selected for presentation at the International Gerontological Congress in Kiev, Russia in July, 1972.

Significance to Bio-Medical Research and the Program of the Institute: The remarkable prevalence (50%) of abnormal glucose tolerance tests in the older population of the United States coupled with the increased morbidity and mortality of patients with diabetes mellitus demands a delineation of the effects of aging on the pathophysiology of carbohydrate metabolism. These studies in man define kinetic parameters in the glucose-insulin homeostatic system and will apply such analysis to patients with disorders in that system.

Proposed Course of Project: Increased numbers of studies are planned on older, diabetic, and obese subjects to utilize the kinetic approach described. Further refining of the insulin-glucose model with regard to control of endogenous glucose production is anticipated along with the description of glucose feedback control on insulin secretion.

Honors and Awards: Dr. Tobin was an invited speaker at the Tuskegee Veterans Administration Hospital symposium on aging in April, 1972.

Dr. Tobin was an invited speaker at a symposium on Current Topics on Aging at the University of Chicago, May, 1972.

Dr. Tobin was an invited speaker at the Southern Sugar Club meeting in Baltimore, September 1971.

Serial No. HD-CP12
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Cytoxan-induced water intolerance

Previous Serial Number: New Project

Principal Investigator: Ralph A. DeFronzo

Other Investigators: Paul J. Davis**
Michael Colvin*
Hayden Braine*

Cooperating Units: Baltimore City Hospitals
* Oncology Service
**Endocrine Service

Man Years:
Total: 0.30
Professional: 0.30
Other: 0.00

Project Description:

Objectives: High dose cytoxan therapy (30-120 mg/kg/day) has been employed with increasing frequency in organ transplantation and in the treatment of neoplasia. With these high doses a syndrome of weight gain, hyponatremia, and decreased urine output with high urine osmolality has been observed. The goals of this study are to determine the frequency with which this syndrome occurs and to define the underlying mechanism.

Methods Employed: Twelve studies were performed in nine consecutive patients. All had normal renal function and excreted a water load normally prior to cytoxan administration. All subjects received 3 liters of normal saline IV for one day preceeding and throughout the period of cytoxan administration (15-100 mg/kg/day). Fluids were permitted ad lib. Urine flow and osmolality and serum sodium and osmolality were followed every four hours; weights were obtained every 6 hours. Because of the possibility of a cytoxan-induced vascular leak, combined ^{95}GRBC volume and ^{131}I albumin space were determined before and during cytoxan administration.

Major Findings: The changes in body weight, urine osmolality and flow,and

serum osmolality and sodium following cytoxan in the seven patients who showed the effect are shown in the following table:

	Weight (kg)	Serum Na (mEq/l)	Serum Osm (mOsm/l)	Urine Osm (mOsm/l)	Urine Vol (ml/hr)
BEFORE CYTOXAN	66.5	139	280	260	207
AFTER CYTOXAN	68.7	130	263	826	70
	P<.02	P<.02	P<.05	P<.001	P<.005

Six to twelve hours following cytoxan there was a striking rise in urine osmolality associated with decreased urine output and weight gain. Serum sodium and osmolality decreased 9 mEq/l and 17 mOsm/l respectively. A close temporal relationship was found between the excretion of cytoxan albylating activity and changes in urine osmolality. Creatinine clearance remained normal. RBC volume and albumin space were unchanged after cytoxan. Three patients were restudied and again showed similar changes. The two patients who showed no effect received the lowest doses of cytoxan (15 and 30 mg/kg). Water tolerance remained normal in control patients who received high dose cytoxine arobinoside (50 mg/kg/day) instead of cytoxan.

Significance to Bio-Medical Research and the Program of the Institute: This syndrome has now been shown to occur in the majority of patients receiving cytoxan and certain new information concerning its mechanisms is now evident. Tumor release of ADH is unlikely because the syndrome was seen in one patient without demonstrable neoplasm (drug induced aplastic anemia), because of the variety of underlying neoplasms (ovarian and lung carcinoma, melanoma, giant cell bone tumor, leukemia) and because of the high frequency with which the syndrome was seen. The close correlation between Uosm and albylating activity suggests that the active metabolite of cytoxan is involved in the mechanism of water intolerance. This could occur by (1) central release of ADH, (2) potentiation of the effects of ADH on the renal tubules, or direct effect on the renal tubule.

Once the pathogenesis of the water intolerance has been delineated, specific therapy might be instituted to prevent the water retention that at times has resulted in congestive heart failure, especially in elderly patients with compromised, compensated cardiac function. The maintenance of urine flow would also protect against hemorrhagic cystitis which is believed secondary to local accumulation of cytoxan metabolites with direct toxicity to the bladder epithelium.

Proposed Course of Project: Studies examining the effect of cytoxan and its active metabolite(s) on the toad bladder are planned with Dr. Erwin Singer (University of Pennsylvania Hospital). Studies with ethanol, an inhibition of ADH, are planned to obtain additional evidence concerning the mechanism of the antidiuretic effect and to see if the syndrome can be prevented.

Honors and Awards: None

Publications: None

Serial No. HD-AG21(c)
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Endocrinology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The renin-angiotensin system. I. Chemical-radioisotope derivative assay of plasma renin and angiotensin. II. Plasma renin-plasma aldosterone interrelationships during aging in man. III. Labeled polymeric substrates for renin and related enzymes. IV. Inhibitors of renin: Leucine-Leucine containing peptides and proteins.

Previous Serial Number: HD-AG21(c)

Principal Investigators: Nicholas M. Bath (Resigned 3/31/72)
J. Howard Lutz
Robert I. Gregerman
Robert J. Workman

Other Investigators: None

Cooperating Units: Department of Pediatrics
Johns Hopkins University School of Medicine

Baltimore City Hospitals

Man Years:

Total:	5.35
Professional:	5.35
Other:	0.00

Project Description:

Objectives: The protease of renal origin, renin, is responsible for the formation of the decapeptide, angiotensin I, by the cleavage of a specific plasma glycoprotein. Angiotensin I is degraded and activitated during passage through the lung with the production of the octapeptide, angiotensin II. This peptide, the most powerful pressor agent known, is believed, under physiologic conditions, to be an important controlling agent of the synthesis of the major regulator of mineral metabolism, aldosterone, in the adrenal cortex. Under pathophysiologic circumstances renin and angiotensin excess are thought to be closely related to the genesis of renovascular hypertension. The precise and specific assay of renin has continued to receive attention because of the following considerations. First, although a direct role of renin in the pathogenesis of renovascular hypertension has very recently been challenged, the deter-

mination of renal vein renin by bilateral catheterization is at present the most definitive means of detecting hypertension due to unilateral reduction of blood flow. Since a ratio of 1.5 between the kidney of the two sides is diagnostic and presents a criterion for surgical correction, a high degree of precision of any assay method is necessary. Second, a sub-group of patients with "essential" hypertension, approximately 25 percent of the total hypertensive population, has suppressed renin. The cause of this suppression is unknown, but these individuals appear to suffer a greatly reduced incidence of cardiovascular morbidity. Renin itself is thought to be vasculopathic, and this might explain the reduced morbidity of low-renin hypertension. Identification of these individuals appears to have therapeutic implications.

Our laboratory has been concerned with original approaches to the measurement of renin and angiotensin and to measurements of the renin-angiotensin-aldosterone system in normal and pathologic aging.

Methods Employed: Our first effort in this area was the development and evaluation of a double isotope derivative method for peptides and specifically for angiotensin I. This approach was then used for renin measurement. Previous reports have presented these efforts in detail.

An entirely different approach has been developed in which polymeric peptide substrates are synthesized and radioactively labeled for extremely simple and rapid assays of the enzyme.

Major Findings: I. The double isotope assay for angiotensin I was previously reported as having been applied to the assay of renin in human plasma. The process of data gathering has now been essentially completed in a series of normal subjects under varying conditions of salt loading and after diuretic (furosemide) administration. The data are undergoing statistical analysis and will soon be prepared for publication.

This assay approach uses a protein substrate prepared from hog plasma. It has been found that a standard amount of renin produces different rates of generation of angiotensin I with different batches of substrate. This observation along with others suggests that the plasma derived substrate contains variable amounts of a renin inhibitor. Other workers have proposed that the quantity of angiotensin I generated per unit time with excess substrate be used as an international means of expressing enzyme activity. Our observations indicate that such an approach is premature, and that it remains necessary to express plasma activity in terms of an international standard of enzyme. Perhaps more important will be elucidation of the nature of the inhibitor.

All remaining methodologic work with the double isotope assay will be terminated within a few months. However, during that time additional comparisons will be made between the double isotope assay results and those of bioassay. We have already performed one such comparison in collaboration with Dr. Anne B. Gould (Cleveland). The relationship between angiotensin generated in plasma and assayed by both methods was only fair. The coefficient of correlation (r) was approximately 0.8 ($p < .01$) but

numerous samples were in gross disagreement. Samples have been secured for a new comparison of bioassay and double isotope assay. This comparison should be definitive. The samples have already been bioassayed twice by independent laboratories with good agreement.

Preliminary work is under way to explore the use of the non-radioactive dinitrophenyl derivative of angiotensin I for renin assay. This approach uses the same methodology and chemical derivative of angiotensin I coupled with a new high speed liquid chromatography technique and a very sensitive nanogram range ultraviolet detection system. Since this approach can potentially be automated, it holds some promise as an alternative to the much more complicated isotope derivative approach. No major effort is planned in this regard, but,if preliminary experiments are encouraging, further work may be done.

Another potential use of the dinitrophenyl derivativazation method is in the detection of sequence failures in peptides prepared by solid phase synthesis. In this connection comparison of purified synthetic angiotensins is being made by direct conventional chromatography and by analysis of the dinitrophenyl derivatives. Preliminary results suggest that dinitrophenylation of peptides may be a powerful analytical tool for the detection of closely related synthetic peptides in allegedly pure materials.

II. We have invested considerable time and effort to establish a working method for angiotensin I by immunoassay. The motives here were for both analytical and physiologic purposes. First, we wished to compare the results of double isotope assay (and bioassay) with that of the now in general use immunoassay. In part this represented an effort at mutual validation of these various techniques, but in addition it was felt worthwhile to evaluate in critical form the most widely used commercial assay system for measurement of renin. Our work has convinced us that the commercial "kits" (available from Schwartz BioResearch) are inferior to other immunoassay materials. Others have simultaneously arrived at the same conclusion. Blanks are high and variable, and precision is poor. The materials supplied appear to vary from time to time with poor quality antibody and/or ^{125}I-angiotensin. We abandoned this approach and have successfully established an immunoassay system using a much more satisfactory antibody obtained from Dr. Werner Waldhausl (Vienna). This will allow us to make the desired comparisons with double isotope assay but, more important, will allow us to perform certain physiologic measurements in elderly individuals.

For the physiologic studies we have established a working relationship with Drs. Avinoam Kowarski and Claude Migeon of the Department of Pediatrics, Johns Hopkins University. We have begun to measure quantitative aspects of response of young and elderly subjects in terms of the interrelationships between renin release and plasma aldosterone responses (immunoassay) for aldosterone.

III. In our previous report we described the synthesis of a new labeled substrate for renin: N-acetyl-poly-L-glutamyl (M.W. 50,000)-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser labeled with ^{125}I. This material

is an excellent substrate for renin and pseudorenin in buffer systems. Kinetic data were previously described. That work has now been submitted and accepted for publication. Further studies on the cleavage of this substrate by human plasma show that, although the rate of cleavage is 10 to 30 times that which can be accounted for by renin, as measured by double isotope assay, the cleaving activity is statistically correlated with that of renin ($r = 0.7$; $p = .01$). This finding suggests that the pseudorenin activity of plasma (which appears to account for plasma's activity) is of renal origin. Previous work on pseudorenin was performed with a tetradecapeptide substrate and suggested no relationship between renin and pseudorenin. The difference of substrates is probably responsible for the new finding. These data are being prepared for publication.

Since renin is probably released from a specialized lysosome, the juxtaglomerular Bowie-positive granule,it is perhaps not remarkable that its release should be paralleled by the release of other proteases. This consideration suggests that parallel measurements of plasma renin and pseudorenin (using the new labeled substrate) under conditions where renin activity is known to vary should be most interesting. Similarly, isolation of the juxtaglomerular granules may be possible by a newly described technique with use of our labeled substrate as a rapid means of following the enzymes. Isolation of the granules would allow determination of their other enzymatic activity in detail and might reveal a variety of new physiologic considerations.

Work has progressed on the synthesis of a new polymeric renin substrate. This material may prove to be a more specific substrate which will, hopefully, not be attacked by pseudorenin. The new substrate is carboxyl-blocked poly-L-lysine to which has been coupled the C terminus of Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser. This material has been labeled with ^{125}I. Enzymatic cleavage has not yet been studied but should yield the ^{125}I nonapeptide Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu (des-aspartyl-angiotensin I) in contrast to the poly-L-glutamyl substrate which yields Leu-Val-Tyr-Ser. A method has been devised for the preparation of the expected labeled nonapeptide product. This work is in progress.

Great difficulty in coupling of the tridecapeptide Arg....Ser to poly-L-lysine was encountered. This was due to a combination of solubility and reactivity problems. However, a method of coupling was discovered in which the trifluoroacetyl salt is soluble in dimethylformiamide allowing a non-aqueous coupling reaction with dicylohexylcarbodiimide. All efforts to obtain satisfactory coupling with water soluble carbodiimides were unsuccessful or resulted in apparently cross-linked polymers which were insoluble. The use of the trifluoroacetyl salt of poly-L-lysine for the coupling of minute amounts of smaller peptide to the polymer should be of more general interest, especially for the production of specific antibodies to synthetic or natural peptides.

IV. In our previous report we described the inhibition of the cleavage of the poly-L-glutamyl substrate for renin by plasma. In our effort to identify the nature of the plasma protease inhibitor we undertook several control experiments in which a variety of proteins were tested as potential

inhibitors of the enzyme. We wished to be certain that the inhibition was not a "non-specific" protein effect. It was soon realized that while several purified proteins (e.g. albumin and 7 S gamma globulin) produced no inhibition, thus ruling out a non-specific protein effect, one of the choices, hemoglobin, was surprisingly a potent inhibitor. Further study of this phenomenon indicates that both purified human and horse hemoglobin's are inhibitory. Furthermore, horse globin is also inhibitory. Inspection of the known amino acid sequence of hemoglobin reveals several Leu-Leu sequences. A working hypothesis has been developed which holds that the inhibition by hemoglobin is due to these Leu-Leu sequences which act either as competitive sites or as alternate substrates. Kinetic studies show that hemoglobin is, indeed, a competitive inhibitor of renin. Further study is necessary to identify the inhibitory sites and to determine if they are being cleaved. If so, our concept of renin as a specific Leu-Leu peptidase will have to be revised.

The sequence of globin horse hemoglobin is most favorable for study. α and β chains of horse globin have been prepared by carboxyl methyl cellulase ion-exchange chromatography. Each chain will be split by cyanogen bromide to yield two peptides. In each case one of the two products will contain one or more Leu-Leu sequences. If inhibition is shown only by the fragments with Leu-Leu bonds, our hypothesis will be strongly supported. We already know that the cyanogen bromide cleavage mixture is at least as inhibitory as globin itself.

The relationship of these observations to the nature of the inhibitor in plasma is unclear. However, we now have the entire problem of the specificity of renin as a Leu-Leu peptidase under reconsideration. With our rapid polymeric substrate assay it has been possible to screen other Leu-Leu compounds for inhibitory effects. Several very potent inhibitors have already been found. Testing of these compounds is being considered for other assay systems and for in vivo testing in animals. The discovery of simple peptide inhibitors of renin opens the possibility of purification of renin by affinity chromatography.

Significance to Bio-Medical Research and the Program of the Institute: Hypertension is an age-related disease complex which affects millions of persons and inflicts enormous morbidity, mortality and economic losses. The development of techniques which affect the diagnosis and therapy of this process and the progress of research which is related to our understanding of the disease mechanisms is essential to an attack on this problem. In addition, this work will hopefully advance our understanding of the normal, age-related physiologic mechanisms which are involved in the regulation of enzyme release from the kidney, peptide production and the control of the secretion of vital adrenal hormones.

Proposed Course of Project: As described above, work is underway which involves simultaneous comparison of the results of several renin assay techniques. Derivatization of peptides is being evaluated for analytical purposes. Physiologic observations will be made on the interrelationship of plasma renin and pseudorenin using a new labeled renin substrate. The synthesis of additional polymeric substrates will be pursued. The influ-

ence of age on the secretion of renin and the interrelationship of renin to the release of aldosterone will be studied in a collaborative effort with investigators at the Johns Hopkins University. Initial study will include a group of young (less than 30) and elderly (over 60) men. The time course of plasma concentration of these substances will be studied with the stimulus of upright posture in order to determine the effect of age and the role of renin as a stimulator of aldosterone. Studies are planned in which drugs known to abolish the plasma renin response will be used to ascertain their effect on the expected response to upright posture of plasma aldosterone. To date no measurements are available in the literature for normal aged subjects of either plasma renin or plasma aldosterone. This study should help fill an important gap in our understanding of this important area.

Honors and Awards: Dr. Gregerman was an invited speaker at the 3rd American Peptide Symposium, Boston, Massachusetts, on June 18, 1972. He spoke on "Isotope derivative assay of nanogram quantities of angiotensin I: Use for renin measurement in plasma".

Dr. Gregerman was an invited speaker at the Johns Hopkins Department of Pediatrics, January 20, 1972 where he spoke on "The renin angiotensin system-New analytical approaches".

Publications: None

Serial No. HD-AG 22(c)
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Endocrinology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Relationship of age to thyroid function and thyroid hormone metabolism

Previous Serial Number: HD-AG 22(c)

Principal Investigators: Robert I. Gregerman
Barry S. Handwerger

Other Investigators: Paul J. Davis*

Cooperating Units: *Baltimore City Hospitals

Man Years:

Total:	1.00
Professional:	1.00
Other:	0.00

Project Description:

Objectives: The rate of metabolism of thyroxine, a major hormone of the thyroid gland, undergoes a progressive slowing with age. This phenomenon, which was originally described in this laboratory and has since been confirmed elsewhere, has a mechanism which remains obscure. In an effort to understand the basis of this age-related metabolic event we have, over a number of years, directed our efforts toward answering the following questions:

(1) What are the factors which control the rate of thyroxine disposal?
(2) What specifically is the role of hormone transport?

In the past year we have conducted studies on the hormone transport system with particular reference to two aspects: the extracellular transport of triiodothyronine and the nature of the intracellular hormone binding proteins. The latter studies are an extension of earlier work from this Section in which we described intracellular hepatic binding proteins for thyroxine and triiodothyronine.

Methods Employed: Binding studies utilize the techniques of equilibrium dialysis, gel electrophoresis, isoelectric focusing, and a variety of column chromatographic separation procedures. Tissues are prepared by perfu-

fusion to remove extracellular binding proteins. The dog has proved the most suitable animal for our purposes.

Major Findings: In the past year we have found that, contrary to earlier concepts, thyroxine binding prealbumin (TBPA) in man is actually a carrier for triiodothyronine as well as for thyroxine. Evidence from experiments with inhibitors suggest that the binding sites for T_3 differ from those that bind T_4.

The intracellular binding protein for thyroxine and triiodothyronine has been purified and further characterized. A number of organs have been studied. The kidney and small intestine contain a discrete binding protein much like that which we had studied in the liver. Evidence is accumulating to indicate that in addition to the easily observed slow ("S") binder, that a fast ("F") binder is also present, although its nature remains elusive. It has not yet been established that "F" is a protein. "S" has a molecular weight of 60,000-70,000, a pI (isoelectric) of 4.5, greater affinity for T_4 than T_3 and, in the kidney, a binding capacity of 200-600 mcg T_4/mg cytosol protein. The binding sites are not affected by agents which are active against the plasma binding proteins. Dog ovary has a binding protein which is electrophoretically discrete from S and F.

Significance to Bio-Medical Research and the Program of the Institute: This work relates itself to the basic mechanisms of hormone function and action. In analogy with the "receptor" proteins now recognized for steroidal hormones, it is possible that the intracellular binding proteins for thyroid hormones play a similar role. Definition of the character and functional significance of the intracellular thyroid hormone binding proteins will allow us to place in perspective the importance of this material for the age-related alteration of thyroid hormone metabolism.

Proposed Course of Project: Further studies will continue to allow purification and isolation of the intracellular thyroid hormone binding proteins. If possible, a reexamination of the rat as an experimental animal will be attempted, since aged rats are readily available. Studies are planned on the effect of hormones, and certain drugs known to affect thyroid hormone metabolism, on the intracellular binding protein patterns in young and old animals. This study will be an attempt to define the functional importance of the binding proteins and their role in age related hormone metabolism.

Honors and Awards: None

Publications: Davis, P.J., Handwerger, B.S., and Gregerman, R.I.: Thyroid hormone binding by human serum prealbumin (TBPA). Electrophoretic studies of triiodothyronine-TBPA interaction. J. Clin. Invest. 51: 515-521, 1972.

Spaulding, S.W. and Gregerman, R.I.: Free thyroxine in serum by equilibrium dialysis: Effects of dilution, specific ions and inhibitors of binding. J. Clin. Endoc. and Metab. 34: 000, 1972.

Davis, P.J. and Gregerman, R.I.: Separation of thyroxine-binding proteins in human serum at pH 7.4. II. Effect of pH and temperature on the binding capacities of thyroxine-binding globulin (TBG) and thyroxine-binding prealbumin (TBPA). J. Clin. Endocr. and Metab. 33: 699-708, 1971.

Serial No. HD-CP 11(c)
1. Gerontology Research Center
2. Clinical Physiology Branch
3. Endocrinology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Lymphocyte responses to specific thyroidal antigens and the pathogenesis of hyperthyroidism (Graves' Disease)

Previous Serial Number: HD-CP 11(c)

Principal Investigators: Barry S. Handwerger
Robert I. Gregerman

Other Investigators: Paul J. Davis*
David Brandes*

Cooperating Units: *Baltimore City Hospitals

Man Years:
Total: 0.40
Professional: 0.40
Other: 0.00

Project Description:

Objectives: Hyperthyroidism (Graves' Disease) is a process which is associated with the production of LATS (long acting thyroid stimulator), 7-S gamma globulin. This antibody-like material has been thought to arise from activation of the antibody forming tissues by an antigen from the thyroid, the antigen presumably arising as a result of a self-destructive process within the thyroid gland. The 7-S gamma globulin is similar in its action to thyrotropin (TSH).

It is now known that lymphocytes of patients with hyperthyroidism produce LATS during in vitro culture following activation with the non-specific transforming material, phytohemaglutinin (PHA). It is also known that lymphocytes from normal, sensitized individuals (e.g. those who are tuberculin positive) can be stimulated by addition of specific antigen (e.g. tuberculin) to their lymphocyte cultures.

The object of this investigation was to attempt to identify specific antigens within the thyroid which could stimulate the production of LATS in lymphocyte cultures from patients with Graves' Disease.

Methods Employed: Lymphocytes from normal individuals were grown in

short-term culture by a standardized technique. Non-specific stimulation was performed with PHA and the response assessed by assay of ^{3}H-thymidine incorporation into the DNA of the lymphocytes. This established that the system was satisfactory for our purposes. Thyroid tissue was obtained from surgical and necropsy material and fractionated into soluble, mitochondrial, microsomal, and nuclear fractions. These fractions were then tested for their effect on normal and Graves' Disease lymphocytes.

Major Findings: The various thyroid fractions had either no affect or a decidedly inhibitory effect on the lymphocyte cultures. In no case was stimulation observed. A variety of maneuvers failed to affect the results. Those included careful attention to sterile technique, Pasteurization, inhibition of proteases and removal of dialyzable toxic components. At this point we became aware of the simultaneous efforts of another laboratory (McGill University) to work with the same concept and a similar lymphocyte system. When it became obvious that they too could see only inhibition of lymphocytes, we decided to abandon the project.

Significance to Bio-Medical Research and the Program of the Institute: The development of auto-antibodies is an age-related phenomenon. Some have held that aging itself may be an auto-immune process. Thus, an advance in our understanding of Graves' Disease, a good example of an auto-immune disease, would have possibly shed some light on our knowledge of auto-immunity and aging. Unfortunately, the approach used here, though simple in performance and attractive in concept, seems to be unworkable for technical reasons which are not presently understood.

Proposed Course of Project: All work has been abandoned until such time as more information is available about factors influencing the lymphocyte culture system.

Honors and Awards: None

Publications: None

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Gerontology Research Center
Laboratory of Cellular and Comparative Physiology

During the past year the Laboratory of Cellular and Comparative Physiology focused on studies to define the relationship between lysosomal activities and the aging process.

Age-related changes in the activities of multiple forms of acid phosphatase in the rotifer have been estimated qualitatively. Polyacrylamide gel electrophoresis indicates the presence of 8 isozymes. Of these, 5 remain constant throughout the lifespan, whereas one decreases and two increase in activity. The activity of the isozyme which undergoes the largest change concomitant with aging becomes most marked at the cessation of reproduction, and continues to increase throughout the post-reproductive period, coincident with the onset of high mortality rates. These observations are consistent with the hypothesis that aging in the rotifer is programmed rather than stochastic in pattern.

Lysosomal function in the left ventricle of 6, 12, and 24 month old male rats has been monitored by electron cytochemical studies of acid phosphatase, β-glucuronidase, aryl sulfatase, and cathepsin B activities. These histochemical studies suggest that (1) the mechanism for lysosomal formation is the same in all age groups; (2) the distribution of lysosomes in the tissue as well as their number do not vary with age; (3) the amount of mitochondria subject to degradation by lysosomes seems to be constant with age; and (4) the mechanisms of mitochondrial degradation by lysosomes do not differ with the age of the rat.

Ultrastructural studies of the papillary muscle in the left ventricle of the rat indicate a marked increase in the number of residual bodies as a function of age. The changes are evident by the twelfth month of age and they are significantly abundant at 24 months. Lysosomal destruction of entire papillary myocardial cells is indicated. The extent of this degradation may suggest drastic alteration in valvular function with age.

Serial No.: HD-CCP6

1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Environments and Genetics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Age changes in organ biochemistry associated with dietary alterations

Previous Serial Number: Same

Principal Investigator: Salvatore Leto (100%) (Left 11/14/71)

Other Investigator: Charles H. Barrows, Jr. (25%)

Cooperating Units: None

Man Years:

Total:	2.75
Professional:	0.75
Other:	1.50

Project Description:

Objectives: Nutrition has a well established role in the aging process. This investigation is part of a program which has addressed itself to the question of the contributing role played by nutrition in this biological phenomenon. A clarification of the role of nutrition in senescence is sought by the application of dietary alterations on experimental animals and subsequent examination of selected organs and tissues by biochemical means. Possible related physiological variables are also being investigated in these animals as a function of chronological age. Specifically, this investigation during the fiscal year has included the continuation of a study of the effect of dietary protein and age on the enzymatic activity and skin collagen extractability of $C_{57}BL/6J$ female mice as well as the effect of diet on protein turnover in several organs of these experimental animals.

Methods Employed: Assay methods have been previously described. The diets used are synthetic diets supplied by General Biochemicals, Chagrin Falls, Ohio modified to our specifications. Turnover measurements were made using standard tracer techniques.

Major Findings: The 50% mortality occurred at 23.5 months in animals fed the control diet (26% protein) and at 28.5 months in those fed the

experimental diet (4% protein). A comparison of the activities of six enzymes per unit of DNA in liver, four in kidney and three in heart, made at six different ages throughout life, showed 10 of the 13 were lower, 2 were the same and 1 was higher in experimental as compared to the control animals. It has been suggested that aging results from continued use of certain genes with a loss of or imperfections in transcription of information. Therefore a reduced rate of use of informational sites would result in the delay of senescent changes. Assuming that low enzymatic activities per unit of DNA indicate reduced rates of transcription and translation, the data of this experiment support this proposal. Furthermore, in two preliminary experiments, the protein turnover estimated by the rate of disappearance of L-arginine (guanido-C^{14}) from liver, kidney, heart and muscle was lower in both 7 and 18 month old experimental animals than in controls.

Significance to Biomedical Research and the Program of the Institute:
Despite marked differences in life spans of many organisms, evidence continues to accumulate indicating that the pattern of senescence may be similar in all organisms. By selecting and studying biochemical systems common to different species or strains within a species, we hope to integrate available data into a unified explanation of senescence. Since nutrition has been shown to affect the longevity of all organisms thus far tested, it is felt that this approach may be fruitful in our search toward elucidating biological aging mechanisms.

Proposed Course of Project: This project has been discontinued.

Honors and Awards: None

Publications: None

Serial No.: HD-CCP2

1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Environments and Genetics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Dietary proteins, aging and the genetic code

Previous Serial Number: None

Principal Investigator: Charles H. Barrows, Jr. (50%)

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description:

Objectives: The increased life span of animals fed diets which contain low amounts of proteins suggests the following two possible biological mechanisms. Restricted dietary protein intakes may decrease protein and RNA syntheses which 1) retard the readout of genetic information and/or 2) reduce the use of the genetic code and thereby minimizes genetic imperfections as they may occur with age. Therefore, in this series of experiments, attempts were made to determine 1) the extent to which an inhibitor of protein synthesis, viz., cycloheximide, would retard the development of chick embryos and 2) the degree to which feeding low protein diets to AKR mice, a strain selectively bred so that 90% die of leukemia, would effect the time of onset and incidence of leukemia. For additional experimental data pertinent to these concepts, see HD-CCP6 and HD-AG13.

Methods Employed: Chick embryos were injected with cycloheximide on the 1st or 12th day of incubation. Their staging, activities of various enzymes, concentrations of DNA, RNA and protein were determined at different ages by standard techniques.

Six groups of 30 AKR mice were fed either a diet which contained 26% or 4% protein. Two groups were fed either of these diets throughout life. The other groups were switched from one of these diets to the other at either 3 or 6 months of age. Mortality was determined and the incidence of leukemia established at autopsy by gross and microscopic pathology.

Major Findings: By the 8th day of incubation, 100% mortality was observed at levels of cycloheximide exceeding 0.9 γ per egg. High mortality (50%-67%) occurred prior to this at levels higher than 0.9 γ per egg with all survivors exhibiting anomalies. At the level of 0.9 γ per egg, no mortality or anomalies were found up to the 8th day. At the fourth day of incubation a retardation of embryogenesis was observed in that the injected embryos were at the 20th stage of development whereas the controls were at the 23rd. Similar but less retardation was observed at levels of 0.75 γ and 0.8 γ cycloheximide per egg. No differences were observed below 0.75 γ per egg.

Changes in the acid and alkaline phosphatases, cathepsins, cytochrome c oxidase and acetylcholinesterase have been established during the 8th to the 17th day of incubation in control embryos. Although, at present, no apparent differences have been observed between control and cycloheximide injected embryos, it is felt that an insufficient number of animals have thus far been examined to justify the conclusion that differences do not exist.

A 27% and 38% reduction in body weight gained was observed on the 19th day of incubation in embryos injected with 5 γ of cycloheximide on the 12th and on the 12th and 14th respectively. No deaths or anomalies were observed in these animals. Furthermore the concentration of DNA, RNA and proteins did not differ by more than 10% among the controls and injected groups of animals.

At 8 months of age the mortality of AKR mice fed diets which contained 26% or 4% protein was 36% and 23% respectively. The mortality in the other four groups were essentially the same as that of the animals fed the 26% protein diet. The incidence of leukemia was 90% or greater in all groups except in the animals fed the low protein diet throughout life in which case it was 55%. Since this study is still in progress, final results and their interpretation can not presently be made.

Significance to Biomedical Research and the Program of the Institute: The ability to control genetic expression by dietary manipulations could result in the retardation of the onset of various diseases and biological aging.

Proposed Course of Project: These studies, still in progress, will be completed during the next year. In addition, the time of release of the virus, which causes leukemia, from the genetic material will be determined by the presence of reverse transcriptase and viral RNA in AKR mice fed normal and low protein diets. Studies will also be carried out in adult rats (16 months) to determine effects of dietary protein levels on mortality and biochemical changes associated with aging.

Honors and Awards: None

Publications: Silbert, J., and Barrows, C. H., Jr.: Cellular senescence and death. In Dikstein, S. (Ed.): The Pharmacology of the Cell. Springfield, Ill., Charles C Thomas, 1972, (in press).
Barrows, C. H., Jr.: Nutrition, aging and genetic program. Amer. J. Clin. Nutrit. In press.

Serial No.: HD-AG13

1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Environments and Genetics Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Aging in the rotifer

Previous Serial Number: Same

Principal Investigator: N. Meadow (100%)

Other Investigator: C. Barrows (25%)

Cooperating Units: None

Man Years:

Total:	2.25
Professional:	1.25
Other:	1.00

Project Description:

Objectives: The rotifer has many characteristics which make it an excellent organism to serve as a model system to study biological aging. Among these are a short lifespan, parthenogenesis and poikilothermia. In addition, it has been reported that nutrition and parental age influence the lifespan of this organism. The general purpose of this program is to determine the physiological, biochemical and histological characteristics of rotifers at various stages of their lifespan and to determine the basic biological mechanisms responsible for senescence and for those factors which influence the longevity of these organisms.

Methods Employed: Specialized techniques for aseptic rotifer culture; and specialized histological techniques for obtaining sections from a few, or even single rotifers. In addition, microchemical, radioisotope assay and biochemical techniques are used.

Major Findings: A study of the multiple forms of rotiferan acid phosphatase was carried out using polyacrylamide gel electrophoresis. The animals were found to contain at least 8 groups of acid phosphatases with a diversity of substrate affinities and pH optima. The study of this complex group of enzymes was facilitated by the development of two new techniques for the development of acid phosphatase zymograms in polyacrylamide gels. The first was a method for the use of p-nitrophenyl phosphate as a substrate. The use of this substrate permitted a direct comparison with the rate of hydrolysis of another substrate, β-glycerophosphate, since the

hydrolysis of both substrates is visualized in the gels by the same reaction: precipitation of inorganic phosphate by lead ion. Thus, relative substrate affinities of the various enzymes could be compared. The second new technique was a method for the analysis, directly in the gels, of approximate pH optima of the various enzymes. It was found that when the pH of the gels was adjusted very thoroughly prior to the development of the zymograms, differences in enzymatic activity could be detected between gels whose pH differed by as little as one-half unit. The procedures required to accurately adjust the pH of gels had not been studied previously.

These methods for the study of multiple forms of acid phosphatase were used to characterize the enzymes present in the bacteria used as food for the rotifers. This study showed that the bacterial enzymes caused only minimal interference with the study of rotiferan enzymes.

Age-related changes in the activity of the rotiferan enzymes were then studied. The specific activity of one of the multiple forms was found to decrease throughout the lifespan, two were found to increase, and five remained unchanged. These findings were interpreted as suggesting that control of enzymatic activity is not lost in old rotifers, as is predicted by several stochastic hypotheses of aging. Rather, the activity of certain enzymes, a finding consistent with the hypothesis that aging is a genetically programmed process. Large alterations in the activity of specific enzymes is considered characteristic of developmental processes. In fact, the initial increment in activity of the acid phosphatase which underwent the largest age-related change occurred at the cessation of reproduction and continued throughout the post-reproductive period. Thus, clear evidence has now been obtained that the cessation of reproduction causes biochemical as well as structural changes in the rotifer, all of which occur coincidentally with the onset of high mortality rates, which were previously found to occur only during the post-reproductive period. This observation is also consistent with the hypothesis that aging in the rotifer is programmed.

The effects on longevity of treatment of the rotifers with low concentration of actinomycin D (0.5-2.0 μg/ml), a known inhibitor of RNA synthesis and cycloheximide (5×10^{-5}-2×10^{-4} M), a known inhibitor of protein synthesis, were tested. These treatments were applied only during the post-reproductive period, since all previous evidence indicated that senescent change occurred only during this phase of the life cycle. The duration of the post-reproductive period was increased in the treated animals by as much as 80% of the control value. These findings constitute further evidence that alterations in gene expression play a role in the aging process.

Proposed Course of Project: The changes in activity of the acid phosphatases reported above do not prove that changes in the quantity of enzymatic proteins are taking place. Therefore, the presence of inhibitors or activators of the enzymes in rotifers of various ages will be tested. In addition, an attempt will be made to radioactively label those enzymes which were found to increase in activity in old rotifers. This would constitute proof that the protein is synthesized during the post-reproductive period.

A determination will be made of the effect of treatment with actinomycin D and cycloheximide on the activity of those acid phosphatases which were found to undergo increments or decrements. This is necessary to show whether control of these enzymes occurs at the transcriptive or translational level. Finally, the alterations in activity of the enzymes will be studied quantitatively by eluting them from gels and measuring their activity in solution. If the enzymes which underwent age-related increments in activity are located in inclusion bodies, an increase in specific activity of over thirty-fold is expected between young and old rotifers.

Honors and Awards: Dr. Meadow was invited to present a seminar to the Biology Department, Johns Hopkins University, Baltimore, Maryland on February 22, 1972.

Publications: Meadow, N. D., and Barrows, C. H., Jr.: Studies on aging in a bdelloid rotifer. II. The effects of various environmental conditions and maternal age on longevity and fecundity. J. Geront. 26: 302-309, 1971.

Serial No.: HD-CCP1
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Morphology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Changes in the cells and extracellular matrices of the crayfish gastrolith disc during growth, development and ageing

Previous Serial Number: Same

Principal Investigator: Dorothy F. Travis (40%)

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.9
Professional:	0.4
Other:	0.5

Project Description:

Objectives: The broad objectives of this program continue to be directed at understanding essentially unknown basic structural and functional principles underlying the activities of dividing, renewable cells as they are associated with synthesis and changes in organization of extracellular matrices during developmental sequences and the ageing process. Specifically, considerations will include principles in both vertebrates and invertebrates which are related to (a) synthesis, organization, orientation, and mineralization of the structural components of the extracellular matrices and to (b) mineral metabolism including mechanisms of storage, release and resorption of inorganic components of skeletal structures.

Methods Employed: The methods being employed for standard ultrastructural studies were generally the same as described in the previous annual report except where mineralized tissues were used. For the electron histochemical studies, the most recent sophisticated methods are being used.

Major Findings: Further changes in structure and function of the gastrolith epidermal cells have been carried out as they synthesize the chitin-arthropodin organic matrix and deposit mineral within the matrix of the young mineralized gastrolith (Stage D_0). Since gastrolith synthesis and mineralization occurs during periods when the animals cease to feed,

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studies are also being carried out on the gastrolith epidermal cells during a stage immediately prior to gastrolith deposition, called the intermolt condition (Stage C_4) which is characterized as one of relative "stability" in the young growing animal. This latter stage is one in which active feeding occurs. In the young animal, it is a stage marked by completion of growth of soft tissues and skeleton, and one in which water content, weight stability, ash content, blood calcium, phosphate and protein, tissue histology and histochemistry show little change until the following growing period. Like other stages of the young growing animal, the intermolt condition (Stage C_4) is cyclic in occurrence up through the fourth year of age. In the fifth year of age, however, this and other cyclic events cease, and the animal ages in this same suspended stage (Stage C_4) of differentiation. This means, in effect, that the gastrolith epidermal cells of the young animal are dividing, renewable cells while in the old animal these same cells become more highly differentiated non-dividing, non-renewable cells, probably resembling in cellular ageing those phenomena seen in mammalian cells of the heart, kidney, adrenals, and in the nervous and other systems.

From these studies, progress has been made in establishing some essentially unknown principles underlying the activities of these renewable cells in younger animals. The most important cellular principles established thus far relate to: 1) mechanisms of transport of the mineral and organic phase across the apical cell membrane into the developing gastrolith matrix; 2) the function of lysosomes during synthesis of the mineralized matrix; changes in mitochondrial localization.

During gastrolith synthesis the transport of the mineral and organic material thus far appears to occur by two different mechanisms. Coated Golgi vesicles containing granular and filamentous material, macromolecules of, or precursors to structural components of the mineralizing gastrolith matrix, are transported to the apical cell surface and their contents are released into the developing gastrolith matrix by reversed pinocytosis. Mineral particles bound to the filamentous material within the Golgi vesicle may have been packaged in the Golgi or it may have been secondarily transported to the vesicles before release. In addition to the filamentous material arising from coated Golgi vesicles and the tips of the microvilli, mineral and organic material are transported across the cell membrane by a second mechanism, which involves the actual release of phospholipid membranous vesicles into the developing matrix. Amorphous appearing mineral particles and sometimes small crystallites are apparent within the vesicles. Though the origin of the vesicles is not as yet clear, they are distinctly analogous to the recently observed matrix vesicles of vertebrate bone and cartilage, which serve as primary nucleating sites in these extracellular matrices. These studies have clearly shown that there are two types of material released from the cell surface into the developing gastrolith, the granular and filamentous material and the vesicular material. These appear to be released in alternating secretory phases, the filamentous in one phase, followed by an alternating vesicular phase. Ordering and compacting of this alternating pulsed material is visualized at more distant sites from the cell surface.

The function of lysosomes during synthesis and mineralization of the gastrolith matrix, periods when the animals do not feed, involves portions of basal breakdown and remodeling of the gastrolith epidermal cells and complete degradation of many of the connective tissue cells containing large accumulations of glycogen. The gastrolith epidermal cells reutilize these breakdown products of proteins, simple and complex lipid and carbohydrates in the synthesis of gastrolith matrix proteins, lipids (phospholipid and sterols), and polysaccharide components. Especially interesting with regard to sterol synthesis and possibly phospholipid is the abundant development of smooth surface configurations of the endoplasmic reticulum in the apical regions of the cell. It is probable, though subject to radioautographic proof, that the SER is intimately involved in sterol and phospholipid synthesis from recycled lipid breakdown products. The former constitute about 4% of the gastrolith matrix by dry weight, while the latter constitute about 5% by dry weight.

Mitochondria in the intermolt cells are predominantly localized in tightly packed arrays from midway to near the apical ends of the cells and are rarely found in other regions of the cell. Although this localization was originally thought to be true of cells during early gastrolith deposition (Stage D_o), it is now clear that tightly packed arrays of mitochondria are as frequently observed basally as apically, depending upon whether they were statically fixed at one site or the other during their movement from the base of the cell to its apical regions. It is reasonably clear that this change in mitochondrial localization during early gastrolith deposition must reflect their marked activity in actively transporting recycled breakdown materials across the basal and lateral cell membranes from the blood, for synthesis of components of the gastrolith matrix in addition to their active participation in release of synthesized materials into the gastrolith matrix at the apical cell surface.

Significance to Biomedical Research and the Program of the Institute: Now being established are structural and functional principles of the activities of cells which are synthesizing, organizing, orienting, and mineralizing the structural components of the gastrolith extracellular matrix. These functional principles intimately relate to similar mechanisms that are operative in developing vertebrate bone and cartilage.

Also being established are basic structural and functional principles underlying the activities of dividing, renewable cells in the young animal. These cellular principles now being established will lay the basic foundation for studies of the same cells when they cease to divide, become highly differentiated and non-renewable in the old animal.

Proposed Course of Project: Studies will continue to elaborate further the principles underlying the activities of these skeletogenic cells and their age-associated changes.

Honors and Awards: Dr. Travis was invited to speak at Johns Hopkins University in a research seminar of the Department of Physiological Chemistry.

Dr. Travis was invited to speak at the Santa Catalina Island conference entitled, "The Comparative Molecular Biology of Extracellular Matrices."

Publications: None

Serial No.: HD-CCP8
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Morphology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Age-related changes in mammalian myocardial cells

Previous Serial Number: Same

Principal Investigators: Dorothy F. Travis (40%)
Trexler M. Topping (100%)
Toshihide Sato (100%)

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.9
Professional:	2.4
Other:	0.5

Project Description:

Objectives: The broad objectives of this program continue to be directed at understanding basic structural, functional, and biochemical principles underlying the activities of myocardial cells during developmental sequences and the ageing process. Encompassed in these considerations of highly differentiated, non-renewable cells, studies are being carried out on both the myocardium and papillary muscle cells of the left ventricle.

Methods Employed: Standard ultrastructural and electron histochemical and other modified cytochemical procedures are currently being used on the left ventricular myocardium and papillary muscles of 6, 12, and 24 month old male rats.

Major Findings:
A. The left ventricular myocardial cells. As a background to current findings, previous studies in this laboratory established that there were age-associated increases in the number of residual bodies, reflecting increased lysosomal activity in only two specific regions of the myocardial cell, the mitochondrial cones at the nuclear poles and in the mitochondrial rows separating the myofilament masses. Furthermore, it was established that the residual bodies in rat myocardial cells originate from degradation of two major cell components: (1) the mitochondria and (2) lipid in droplet form. It was shown that lysosomal degradation of mitochondria occurred by

two methods: (a) direct coalescence of mitochondria with primary lysosomes without their prior segregation by cytomembranes from other components in the sarcoplasmic matrix, a previously undescribed mechanism; and (b) by the segregation and digestion in autophagic vacuoles, rarely observed in myocardial cells but commonly observed in other cell types. Lipid in droplet form was degraded to residual bodies after prior segregation by smooth membranous elements of the sarcoplasmic reticulum.

Current studies strongly indicate that a third mechanism of lysosomal degradation of mitochondria occurs in the myocardial cells. In this unusual mechanism, the primary lysosome, showing aryl sulfatase, acid phosphatase and cathepsin B activity, coalesces with the mitochondrial membrane, encircles the mitochondrion and degrades the latter in its internum. This type of degradation has previously been described in only the anterior pituitary thyrotropic, mammotropic, and gonadotropic cells in which primary lysosomes regulate the secretory process by taking secretory granules into their internum and degrading them. This crinophagic mechanism of mitochondrial degradation in the myocardial cell appears to be distinctly different from the direct coalescence mechanism of mitochondria with primary lysosomes. In this latter instance the lysosome coalesces directly with the outer membrane but does not degrade it. The "wave" of hydrolase activity appears to spread along the inner membrane and cristae degrading these components and the mitochondrial matrix leaving the outer membrane intact as a protective membrane to other components in the cytoplasmic matrix.

In attempts to determine the sequence of lysosomal degradation of mitochondria by the mechanisms indicated, the mitochondria were marked by the localization of cytochrome oxidase activity between the inner and outer cristal membranes. However, no images were obtained showing the direct coalescence of the mitochondria with primary lysosomes. On the other hand, in the modified crinophagic mechanism of degradation, there was a loss of cytochrome oxidase activity in the cristal remnants within the secondary lysosome, as might be suspected. It was not possible to determine from these images whether cytochrome oxidase activity was lost as a consequence of, or preceding degradation. Furthermore, there was no difference in the cytochrome oxidase activity in mitochondria from the nuclear pole zones or those in the mitochondrial rows, separating the myofilament masses. Similarly, there were no evident age-associated changes as visualized with respect to stain distribution or intensity.

Following segregation of lipid droplets in old cells from other components in the sarcoplasmic matrix by autophagic vacuole formation, the present studies have clearly established: (1) the presence of aryl sulfatase and acid phosphatase activity in the cap-like matrix of the segregated lipid droplet, and (2) this activity spreads into the lipid droplet participating with auto-oxidation in its degradation to residual bodies.

As visualized by the localization of aryl sulfatase, acid phosphatase and cathepsin B, primary lysosome synthesis transport and packaging in the myocardial cell occurs by two different pathways: (1) In the nuclear pole zone, acid hydrolases are synthesized in the nuclear envelope (part of the RER); vesicles are pinched off from the latter which become incorporated into the

Golgi cisternae and primary lysosomes are packaged and released as single membrane bounded bodies from the swollen end-like sacs of the cisternae. (2) At distant sites from the nuclear pole zone, lysosomal enzymes are also synthesized in the RER but are subsequently transported to the SER where they are ultimately released from the latter as single membrane bounded vesicles (lysosomes) containing tightly packed myelin-like whorls or finely stippled matrices showing acid hydrolase activity. This mechanism of lysosome formation and release could most certainly enable the lysosome enzymes in myocardial cells to participate by a more direct route by avoiding the Golgi complex. Significant in this respect, is the close packing of organelles and masses of myofilaments (between the nuclear pole zone and the periphery of the myocardial cells) which could well impede transport of lysosomes from the Golgi zone to more distant sites within the cell.

These results have also shown that there is little acid phosphatase or β-glucuronidase activity, moderate aryl sulfatase activity and much more cathepsin B activity in the left ventricular myocardial cells throughout 6-24 months of age.

B. The left ventricular papillary muscle cells. Studies in progress have thus far shown that there are marked age-associated changes in the rat left ventricular papillary myocardial cells. These changes are evident by the twelfth month of age and are marked by increases in the number of residual bodies (reflecting increased lysosomal activity) in the mitochondrial cones at the nuclear poles and in the mitochondrial rows separating the myofilament masses. As in previous studies of the left ventricular myocardium, the residual bodies appear to arise from the lysosomal degradation of mitochondria, lipid in droplet form and glycogen. By the twenty-fourth month of age, there are further increases in the number of residual bodies but even more significant, there is marked lysosomal destruction of many of the papillary myocardial cells themselves, a heretofore unreported finding in the normal ageing papillary myocardial cells.

Significance to Biomedical Research and the Program of the Institute: The electron cytochemical studies of the left ventricular myocardial cells are the first to encompass an age span of 6-24 months and the study of the left ventricular papillary muscle cells is the first age-associated study of these cells of any mammal. The function of lysosomes and the mechanisms of their activity in these highly differentiated, non-renewable cells with age are being established. This information is fundamental to the understanding of myocardial function with age.

Proposed Course of Project: Further studies will continue to delineate the basic structural, functional, and biochemical principles underlying the activities of myocardial cells during the ageing process. Extended studies will involve quantitation of age-associated lysosomal and mitochondrial enzymatic activities in the left ventricular myocardial and papillary muscle cells and subcellular fractions.

Honors and Awards: None

Publications: Travis, D. F., and Travis, A.: Ultrastructural changes in the left ventricular myocardial cells with age. J. Ultrastructure Res. 39: 124, 1972.

Serial No.: HD-CCP11
1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Morphology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Ultrastructural studies of the regenerating sea urchin spine during growth and developmental sequences

Previous Serial Number: Same

Principal Investigators: Barry M. Heatfield (100%)
Dorothy F. Travis (20%)

Cooperating Units: None

Man Years:
Total: 1.2
Professional: 1.2
Other: 0

Project Description:

Objectives: Cellular mechanisms underlying synthesis, mineral storage, release, and calcification of extracellular matrices, are being studied throughout developmental sequences and aging. The regenerating sea urchin spine is being used as a model system for these studies because the endoskeleton parallels vertebrate bone in embryological origin and structural organization. Since initially nothing was known about their fine structure, regenerating spines of the sea urchin, Strongylocentrotus purpuratus, were examined by electron microscopy to identify those cells involved in skeletogenesis.

Methods Employed: Modern cytological and cytochemical techniques and procedures of both light and electron microscopy are being utilized.

Major Findings: The tip of the young regenerate consists of two layers of tissue, an outer epidermis and an inner calcified dermis separated by a thin basal lamina. Both the epidermis and calcified dermis are characterized by mixed populations of cells. In all, approximately 10 cell-types have been identified in the young regenerate, four in the epidermis and six in the calcified dermis. The ultrastructure of these cell-types has been studied in detail. They can be divided into those with spherules (secretory bodies) and those without spherules. In the epidermis, there are two types of epidermal cells, possibly representing stages in the differentiation of a single cell-type, and two distinct spherule-bearing cell-types, eleocytes

and morula cells. In the calcified dermis, there are "precalcoblasts," "calcoblasts," and an unidentified cell-type, all of which may represent various stages of differentiation of a single skeletogenic cell-type. Phagocytes and two spherule-bearing cell types, the "granulocyte" and "vacuolecyte" also occur in the calcified dermis, in addition to eleocytes and morula cells mentioned above. The spectrum of functions of cell-types remains to be described.

In terms of the objectives of this project, the most significant cell-type identified in tissues of the young regenerate is the calcoblast. This cell-type is most prevalent in the calcified dermis at the distal tip where it is frequently in intimate association with the growing mineral surface of the skeleton. Calcoblasts are characterized by narrow pseudopodia containing elements of the rough endoplasmic reticulum (RER) and mitochondria. The nucleus has a finger-like protrusion, and contains a single nucleolus and relatively little condensed chromatin. The outer surface of the perinuclear membrane is studded with ribosomes. A well-developed Golgi complex of parallel, wavy arrays of membrane bound cisternae and associated vesicles is disposed around the nuclear finger. The cytoplasm contains single or small clusters of ribosomes, numerous small vesicles of the smooth endoplasmic reticulum (SER), and narrow membrane bound cisternae of the RER which generally parallel the cell surface and contain finely granular or filamentous secretory material. Mitochondria occur throughout the cytoplasm, with typically one or more at the tips of the pseudopodia. The pseudopodial cytoplasm between mitochondria may be partially constricted, and the main body of the cell occasionally contains lipid droplets, myelin figures, multivesicular bodies, and vacuoles with variable amounts of granular or heterogeneous material. Pseudopodia are restricted to that portion of the cell body around the Golgi complex, and thus this cell-type shows a polarization in the distribution of cellular organelles.

Calcoblasts participate in the formation of a cytoplasmic sheath which surrounds the tips and sides of microspines comprising the young skeleton, and may extend to subapical portions of the skeleton. The cytoplasm of the sheath contains numerous small vesicles, and, occasionally, mitochondria, but not elements of the RER or other cytoplasmic organelles. The inner plasma membrane of the sheath frequently displays small evaginations similar to reverse-pinocytotic vesicles. A space of variable width separates the inner aspect of the sheath from the mineral surface, and contains an extracellular amorphous matrix.

As interpreted from the available evidence, growth of the endoskeleton occurs in the following way. The skeletal matrix is synthesized by the RER and Golgi complex and consists of mucosubstances contained in vesicles in the sheath cytoplasm which are released into the extracellular compartment by reverse pinocytosis. Deposition of calcium salts subsequently takes place within this matrix. An analogy therefore exists between skeletogenesis in the regenerating sea urchin spine and osteogenesis of vertebrate bone where collagen and ground substance are initially released and organized into patterns by osteoblasts, followed by deposition of calcium salts relative to this preformed pattern.

Significance to Biomedical Research and the Program of the Institute: The results of these studies have a direct relevance to our understanding of calcifying systems, particularly vertebrate bone, because the available evidence suggests that similar mechanisms are operative in a wide variety of calcified tissues. Moreover, the continuing study of concomitant changes in structure and function of the calcoblast cell-type throughout developmental sequences and aging will be one of the first dealing with such age-associated changes in a calcified tissue.

Proposed Course of Project: The present research has identified the calcoblast cell-type. Studies will now be carried out on mechanisms of synthesis, mineral storage, release and calcification of the skeletal matrix. These studies will involve cytochemical, enzymatic, and radioautographic techniques at the ultrastructural level. Concomitant changes in calcoblast structure and function throughout developmental sequences and aging will then be initiated by comparing the young, rapidly growing regenerate with the old, non-growing regenerate.

Honors and Awards: None

Publications:

Heatfield, B. M.: Growth of the calcareous skeleton during regeneration of spines of the sea urchin, Strongylocentrotus purpuratus (Stimpson): a light and scanning electron microscope study. J. Morphol. 134: 57-89, 1971.

Heatfield, B. M.: Origin of calcified tissue in regenerating spines of the sea urchin, Strongylocentrotus purpuratus (Stimpson): a quantitative radioautographic study with tritiated thymidine. J. Exp. Zool. 178: 233-246, 1971.

Davies, T. T., Crenshaw, M. A., and Heatfield, B. M.: The effect of temperature on the chemistry and structure of echinoid spine regeneration. J. Paleontol. In press.

Serial No.: HD-AG37

1. Gerontology Research Center
2. Laboratory of Cellular and Comparative Physiology
3. Morphology Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on the comparative physiology of aging and environmental effects on the ageing process

Previous Serial Number: Same

Principal Investigator: M. A. Brock (100%)

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Description:

Objectives: Altering the body temperature of homoiotherms or the ambient temperature of poikilotherms results in changes in cellular functions, including those related to development and longevity. Moreover, in certain organisms, there are rhythmic, temperature-independent changes in the rate of ageing. These are exemplified by cyclic changes (both yearly and of shorter duration) in the life span of the hydranths of the marine coelenterate, Campanularia flexuosa. It is the objective of these studies to explore the cellular mechanisms responsible for changes in the rate of ageing.

Methods Employed: Campanularia flexuosa colonies were cultured in aerated, artificial sea water at 4°, 10°, 17° and 24° for ageing studies and other determinations at each of these temperatures. The colonies were mapped daily, and developmental stages and longevity of hydranths were noted for each successive day. Biochemical procedures, including cellulose acetate strip electrophoresis, were employed.

Major Findings: Rhythmic changes in development and longevity were observed in Campanularia hydranths and colonies grown at 10°, 17° and 24°. The persistent rhythms were circannual (about one year) and were themselves comprised of shorter, persistent cyclic changes in longevity of the hydranths. The rate of ageing was also modified in Campanularia by varying the environmental temperature.

A. Circannual rhythms in Campanularia. Changes in development, growth and longevity occurred synchronously in yearly cycles at all ambient temperatures and were characterized by changes in: 1) hydranth longevity; 2) the number of individuals that developed abnormally and never reached the adult stage; 3) the rate of replacement of regressed individuals, a decline in the rate leading to decreased colony size; 4) upright life; and 5) gonophore development. The rhythms in colonies grown at 10° have persisted for over 3 years. At the higher ambient temperatures, 17° and 24°, survival of the colonies was threatened after approximately one year at 17° and 10-11 months at 24°, some rhythms were damped, and the colonies finally died.

(1) Rhythmic changes at 10°, 17° and 24°.

March-May. Maximum hydranth life spans were observed at all 3 temperatures (see Table 1 for representative data). Few or no individuals developed abnormally. The growth of colonies was luxuriant with the addition of many new individuals and prolific branching of the uprights; no uprights died. Gonophores developed on uprights at 17° and 24° during May; none ever formed at 10°.

June-Sept. Minimum hydranth life spans were observed (Table 1); the differences between the Summer minima and the March-May maxima were highly significant at all ambient temperatures ($P < .001$). There was a rise in the number of abnormally developing individuals to 5-6% at 10°, 17-26% at 17° and 33% at 24° (the colony at 24° died at this time). Colony growth was further depressed by the lack of replacement of regressed individuals and by the addition of few or no new individuals. Uprights died at 17° and 24°.

Oct.-Jan. Hydranth life spans increased to near the maxima observed in March-May. The percentage of abnormally developing individuals was low: 4% at 10°, 5-10% at 17° and 3-6% at 24°. Growth of the colonies was prolific with the addition of many individuals and branches. No uprights died.

Feb. A severe repression of colony growth occurred during Feb. at all 3 temperatures. It was the culmination of changes that included: 1) a marked decrease in hydranth life spans to the Summer minima; 2) a sharp rise in the percentages of individuals that never developed to adults, the percentages ranging from 20-30% at all ambient temperatures; 3) lack of replacement of regressed individuals and the addition of few or no new individuals; and 4) upright death. If the colonies survived, this period was followed by the spontaneous initiation of prolific new growth, and hydranth life spans increased to their maxima.

Table 1. Colonies at 10°

Date	N	Mean Life Span (Days ± S.E.)
Jan. 1969	201	16.7 ± 0.4
June 1969	266	7.4 ± 0.1
March 1970	149	16.7 ± 0.5
Aug. 1970	109	9.7 ± 0.3

In summary, in "Winter" colonies, individual life spans were maximal and colony growth expansive. In "Summer" colonies, a decrease in the life spans of individuals was accompanied by a rise in the percentage of abnormally developing hydranths and repression of colony growth.

(2) The period of the circannual rhythm. Using as a marker the repression of colony growth which occurred during February of 1969-1971, the period was slightly over a year, averaging 374 days.

(3) The nature of the circannual rhythm. That the rhythms described are expressions of endogenous genetically controlled events is being further tested by comparing the colonies that have been grown under constant laboratory conditions for over 3 years with colonies obtained from Woods Hole, Massachusetts in November 1971. The new colonies from Woods Hole, exposed to cues from the environment until November 1971, should differ from the long-term laboratory-grown cultures. Using the changes in colony growth and hydranth longevity during February as markers, it is expected that any changes in the new colonies will be displaced temporally from those in the long-term laboratory colonies. Current data tentatively support this.

B. Rhythms in longevity with a period of less than one year. Persistent, temperature-independent rhythms in hydranth longevity were observed in colonies at all ambient temperatures, with periods for two representative cycles of 16 days at 17° and 29 days at 10°. A cycle begins with the development of long-lived individuals; progressively shorter-lived individuals develop with time as the cycle unfolds; and at the termination of the cycle individuals with the shortest life spans develop. For the 16-day cycle at 17°, the average hydranth life was initially 9.3 days as contrasted with 2 days at the end of the cycle. At 10°, the average hydranth life was initially 16.2 days and decreased to 6.3 days on the 29th day. In both cases there is a three to four-fold difference in life expectancy within one cycle. A new cycle begins just prior to the termination of the preceding one; there was an overlap of 3-4 days for the 16- and 29-day cycles described. These rhythms have been seen in 3 different cultures of Campanularia obtained from Woods Hole in 1963, 1968 and 1971, and they have been continuously repeating throughout over 3 years of observations. Analysis of the relationship of these rhythms to the circannual cycles has not yet been completed.

C. Acid phosphatase activity in short- and long-lived hydranths. It was previously suggested from ultrastructural and biochemical observations that the activity of isoenzymes of the lysosomal enzyme, acid phosphatase, could vary during development and aging and furthermore that the variations in activity could differ in tempo or mode in the cells of organisms whose life spans are increased.

The number and intensity of bands (up to 5 bands) representing acid β-glycerophosphatase activity in Campanularia hydranths did increase through ageing and regression during February 1971. At that time of repressed colony growth, the hydranth life spans were short (9.9 days). In contrast, during July and August 1971 while colony growth was expansive and hydranth life spans were long (> 13 days), no more than 3 bands with

only slightly increased intensity were seen. There was suggestive evidence for a shift in the pH optimum of the Band 4 enzyme during regression; such a shift was also noted in February.

Significance to Biomedical Research and the Program of the Institute: Animals whose life spans may be extended either due to rhythmic or induced changes in the rate of ageing are useful for the exploration of the cellular mechanisms related to the increased longevity. With an understanding of the regulatory mechanisms, the possibility exists that such information may be applied in other species with a view toward extension of the optimally functional portion of the life span.

Proposed Course of Project: The rhythmic variations in Campanularia longevity, both circannual and those of shorter duration, will be further analyzed. Correlative biochemical and ultrastructural techniques will be used to explore: 1) the cellular mechanisms associated with the endogenous changes in Campanularia longevity as well as those induced by changes in ambient temperature; and 2) the effects of hibernation on the longevity of golden hamsters and on cell function.

Honors and Awards: Dr. Brock served as Secretary of the Society for Cryobiology.

Dr. Brock was invited to speak at a AAAS Symposium on Cnidarian Growth and Reproduction.

Publications: Brock, M. A.: The effect of ambient temperature on ageing and on lysosomal activity in Campanularia flexuosa. Cryobiology 8: 300, 1971.

Brock, M. A., and Hay, R. J.: Comparative ultrastructure of chick fibroblasts in vitro at early and late stages during their growth span. J. Ultrastructure Res. 36: 291, 1971.

NICHD ANNUAL REPORT
July 1, 1971 through June 30, 1972
Gerontology Research Center
Laboratory of Molecular Aging

The Laboratory of Molecular Aging has made significant progress in developing scientific data and theory basic to the biology of aging. Research efforts, ranging from the examinations of molecular interactions, in vitro, to investigations of the integration and coordination of physiological systems in the intact animal, have focused on control and regulatory mechanisms in adult development and aging.

A chemical model has been developed and evaluated to test the hypothesis that senescence results from errors in the incorporation of amino acids into proteins caused by the mispairing of nucleotide bases during replication, transcription, or translation. It has been found that in the absence of divalent metal ions, poly (I) reacts with only the complementary cytidines in the copolymer (U,C). In the presence of the metals (MgII, MnII, NiII), the poly (I) reacts with both cytidines and uridines. Thus, divalent metal ions do induce mispairing, presumably because they stabilize polynucleotide strand interactions. Without the divalent metals these interactions are weakened and therefore are sensitive to base complementarity.

Progress has continued on the development of a technique for the sequencing of DNA, which contains the genetically determined information for growth, development, and aging. It has been found that the presence of metal ions greatly enhances the specificity of DNAase I, and that different metals produce different specificities. This effect is due to the property of metals to select one of the bases for preferential binding; the bond adjacent to the selected base is not recognized as a proper site for enzymatic attack. A method has been devised for determining whether DNAase cleaves bonds in one chain of the double helix, or whether bonds in both chains are cleaved simultaneously. Results with Cu-DNA show a lack of complementarity, suggesting that the former, single-hit, mechanism prevails.

A technique, dependent on the hybridization of poly (A) to radioactive poly (U), has been successfully developed in order to identify and quantitate message RNA in the presence of bulk RNA. This technique has been applied to show that (1) mRNA levels in blowflies drop by a factor of 10 half way through the adult flies' life span; and (2) RNA-containing viruses which cause cancer can be distinguished from those which do not cause cancer by the amount and length of the poly (A) sequences. Sequences in DNA have been found that are complementary to the poly (A) in RNA. These sequences appear to be present in the double stranded form in chromosomes. New RNA-DNA hybridization conditions have been established wherein virtually all of a 28s ribosomal RNA is converted to a hybrid structure and is largely resistant to ribonuclease. A procedure for carrying out RNA-DNA hybridizations with RNA covalently linked to the filters has been successfully worked out. This system has potential application to the isolation of genes in a pure form and in determining changes during senescence.

The antiviral defense mechanisms of human diploid (WI-38) tissue culture cells have been found to change with age. Interferon is induced by poly (I):poly (C) most effectively in the middle of the in vitro lifespan and its induction falls off sharply before the cells die.

Studies on the mechanism of active transport in renal tubules have advanced significantly and several basic principles have been established. The rate of binding of D-glucose to the isolated luminal (brush border) membrane is dependent on the sugar concentration. Two saturable, 1-2 μM (Site I), and 50 mM (Site II), systems are found. The high affinity sites can bind 1.4 pmoles/mg protein and the Kglucose at Site I is 3 x 10^4 greater than at Site II. Binding of D-glucose, in contrast to the complete transport mechanism, does not require Na^+ and is not inhibited by ouabain. The importance of sulfhydryl groups for transport is indicated by the inhibition of binding by the diuretic, Mersalyl, and N-ethyl maleimide. Phlorizin inhibits competitively at Site I (Ki = 0.2 mM) but non-competitively at Site II. The bindings of sugars exhibit stereospecificity. L-glucose is bound at a rate 20% that of D-glucose, but by a different mechanism. Bindings of the two isomers are mutually independent; additive; L-glucose does not reverse D-glucose binding; and phlorizin does not inhibit L-glucose binding. D-galactose binding is biphasic, indicating high and low affinity sites and having, respectively, 50 and 25% the rates of D-glucose. Common binding sites for D-glucose and D-galactose are indicated by mutual competitive inhibitions. Two binding sites for phlorizin have been found, with Km values of 8 μM and 2.5 mM. Only binding at the high affinity site is Na^+ dependent. Phlorizin binding is inhibited in a competitive fashion by D-glucose. When glucose binding takes place in a Na^+-free system there is no inhibition by phlorizin in the high affinity range, although glucose binding is inhibited by the same concentration of phlorizin in the presence of Na^+. These results are consistent with the concept that phlorizin and glucose are bound at sites near to one another, to account for the mutual competitive types of inhibition, but are bound to separate sites, one Na^+ dependent, and the other Na^+ independent.

Progress has been made in defining the control mechanisms in cerebral metabolism. Hexokinase in brain is associated with mitochondria, in contrast to its location in the cytosol in most other tissues. In brain the enzyme can be reversibly bound and debound, ATP and ADP being effective solubilizers. It has been found that nucleotide debinding is dependent on the ATP:ADP ratio. At a constant total nucleotide concentration, added exogenously, solubilization increased linearly with increasing ratios. At a ratio of 4.8 mM ATP:0.8 mM ADP, the intracellular concentrations in rat brain, 4X as much enzyme was solubilized than at the reciprocal ratio. During oxidative phosphorylation, when ADP is converted to ATP endogenously, the mitochondrial hexokinase becomes solubilized. An 85% increase in the soluble form of the enzyme is found when the physiological concentration of ATP is obtained. This change in compartmentation of hexokinase during coupled respiration, plus the finding that the soluble form of the enzyme is more sensitive to inhibition by glucose-6-P, suggest that the binding and debinding of the enzyme to mitochondria may be a factor in the physiological control of hexokinase activity, and thus the glycolytic rate, in brain.

The hypothesis of age-related alterations in the action of endocrines in regulating intermediary metabolism has been tested. The specific activities of the mitochondrial α-glycerolphosphate dehydrogenase from livers of adult (12-13 months old) and senescent (27-29 months old) hypothyroid rats have been measured at different intervals following the induction of enzyme synthesis by triiodo-L-thyronine (T_3). There are no significant differences between the two age groups in the rates of induction, the maximal levels of the induced enzyme, or the decreases in activity following the attainment of peak activities. These findings suggest that the biochemical machinery for protein synthesis in this tissue in aged animals is in no way impaired, at least in this specific model system, and that the livers of senescent rats are fully capable of responding to direct adaptive stress.

Changes in the State 3 (ADP-stimulated) and State 4 (controlled) rates of succinate oxidation in liver mitochondria induced by thyroid hormone has also been examined in adult and senescent rats. Adult animals made hypothyroid by feeding thiouracil have significantly decreased State 3 rates of respiration and respiratory control ratios. The State 4 rate of oxidation is not lowered significantly. A single injection of T_3 to these hypothyroid rats results in increases in States 3 and 4 rates as well as in the respiratory control ratios. The enhancement is evident at 6 hours after hormonal administration and reaches a maximum in about 2 days. No differences in the rates of induction nor in the maximal levels achieved are found between mature and aged animals. These findings also indicate that the livers of senescent rats respond fully to direct stimulation by thyroid hormone.

Serial No. HD-GMA1
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Interaction of Metal Ions with Polynucleotides and Related Compounds

Previous Serial Number: Same

Principal Investigators: P. Clark (10% time)
G. Eichhorn (35% time)
J. Rifkind (40% time)
Y. Shin (30% time)
R. Srivastava (100% time)(E.O.D. 1/17/72)
E. Tarien (100% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	3.75
Professional:	3.15
Others:	.60

Project Description:

Objectives: (1) To understand the biological activity of metal complexes of nucleotides and polynucleotides on the basis of their structures and reactions. (2) To find specific reactions of the individual nucleotides. (3) To understand the biological significance of metal ions in nucleic acid function.

Methods Employed: The interaction of metal ions with polynucleotides and their monomeric components is studied, using a variety of physical chemical techniques, e.g., optical rotatory dispersion, nuclear magnetic resonance, ultraviolet and infrared spectrophotometry. From these data the site of interaction of the metal can be correlated with its effect on the reactions of the nucleic acids.

Major Findings: A. Matching of bond distances between pharmacological reagent and biological substrate. The reaction of cis[Pt^{II}en Cl_2] with DNA and poly(A). Recently complexes of platinum have become important because they have been shown to be potent agents in tumor regression, and they have

had successful clinical application in humans. It has also been demonstrated that the most significant biological activity of these substances is the inhibition of DNA replication. It is of interest therefore to investigate how this inhibition occurs. The following hypothesis is proposed: The pharmacologically active complexes [e.g. cis[Pt^{II}en Cl_2], cis-dichloro-ethylene-diammine-platinum(II)] in the solid state form stacks in which the planar platinum molecules are stacked above each other, with a separation of 3.4 Å between them. Since this separation is identical to the separation of bases in DNA, it is proposed that DNA molecules induce the stacking of platinum complexes in a manner similar to that observed in the solid state. The close association of the DNA strands with the stacked platinum complexes would obviously prevent DNA replication.

The interaction of cis[Pt^{II}en Cl_2] and DNA was studied using UV and CD spectra. The CD spectrum of native DNA has a characteristic conservative circular dichroism. The addition of the platinum complex has a profound influence on the conformation of the DNA, as indicated by a non-conservative CD band and a shift in the UV absorption peak, revealing a perturbation of the stacked configuration of the DNA that can be explained by a tilting of the bases to accommodate the stacked Pt molecules. These effects require considerable time. The stoichiometry of the complexes in solution at various intervals of time was determined by continuous variation techniques, using the CD spectrum. After 12 hours, a 2:1 Pt/DNA complex is the principal species in solution; after 24 hours the principal species is a 1:1 complex. The postulated association between a DNA strand and a stacked platinum complex would require the 1:1 complex and it is reasonable that considerable time is required to reorganize both the DNA and the Pt complex into the most stable conformation for the association. In the presence of an excess of KCl, the reaction of the platinum complex is severely inhibited, requiring weeks to attain a state reached in days in the absence of KCl. This phenomenon indicates an inhibition or the displacement of the chlorine by water molecules, so that the active species reacting with DNA must be cis[Pt^{II}en $(H_2O)_2$].

The reaction of the platinum complex with double stranded and single stranded poly(A) was also studied. In both cases dramatic perturbations in the structure were observed, and a continuous variation study revealed a 1:1 Pt/poly (A) complex.

Preliminary evidence indicates that platinum complexes that do not have antitumor activity also do not costack with DNA. These observations are all in line with the hypothesis that costacking of Pt complexes with DNA accounts for antitumor activity.

B. <u>The differentiation between ribosides and deoxyribosides by copper(II) acetate</u>. This previously reported reaction represents a second example of a reagent designed to react specifically with a biological substrate, by matching of bond distances. Copper acetate in non-aqueous solvents is a dimer (Cu_2 acetate$_4$) containing a copper-copper bond bridged by the four acetate groups. This substance reacts specifically with ribonucleosides because the copper-copper distance ($\sim$ 2.7Å) is identical with the distance

between the 2' and 3' oxygen atoms of ribonucleosides; deoxynucleosides lack the 2' oxygen. This reaction has now been subjected to standardization curves to determine its usefulness for the quantitative determination of ribonucleosides. It is useful for quantitative analysis under favorable conditions; the presence of more than one ribonucleoside precludes quantitation. The reaction is very useful for distinguishing qualitatively between the two types of compounds.

C. Metal ions as agents for the stacking of nucleotides. If DNA can be an agent for stacking platinum complexes, metal ions in turn can cause the stacking of mononucleotides. It was previously shown by NMR that Cu(II) can stack 3' and 5'AMP and by ORD studies that zinc(II) can cause the same nucleotides to stack. It is curious that the stacking by copper(II) indicated by the NMR could not be confirmed by ORD. The impasse was resolved by titration studies of 3' and 5'AMP with copper(II) at low (.001M) and high (.05M) concentration. At the low concentration (at which ORD studies are carried out) the titration behavior is characteristic of a non-stacked complex (one OH^- is attached to the metal at a pH lower than for uncomplexed copper, and one OH^- at a much higher pH). At the high concentration the titration is characteristic of a stacked complex. Nickel(II) AMP is titrated as an unstacked complex even at the high concentration, thus, it is established that metal ions are instrumental in stacking mononucleotides, but different metal ions require different concentrations to do this.

D. Metal-nucleotide complexes. (1) The AMP-Pb complex. It has been previously shown that Zn(II) produces a very specific complex with 5' or 3'AMP in which the bases are held in a parallel stacked configuration. We have now shown that Pb(II) also produces a highly rotatory 5'AMP complex in which the bases however are not held in a parallel stacked configuration. Unlike the Zn-complex, the Pb-complex seems to be specific for the 5'-ribonucleotide, i.e., 3'AMP and 5'dAMP do not form such a complex. In other respects the complex seems to have characteristics similar to those of the zinc-AMP complex. As found for zinc, the complex lowers the pK of the secondary phosphate of the nucleotide and raises the pK for the hydrolysis of the metal. The base specificity, to the extent that it has been studied, is also similar to the zinc-complex, in that the adenine moiety is required for complex formation.

(2) Complexes of Cu(II) and Mn(II) with IMP and poly I. Nuclear magnetic resonance studies have revealed that 5'-inosinic acid can bind to Cu(II) at both N-1 and N-7 positions, and that one position or the other may be favored by changing pH. At low pH N-7 is preferably bound, and at high pH N-1 is preferred; around neutral pH both sites have an equal tendency to bind copper. This movement of copper from N-7 to N-1 with increasing pH in 5'IMP is similar to the movement previously observed with inosine, but the transition occurs at much higher pH, reflecting greater stability of the N-7 binding site. This greater stability can be explained by a dimeric complex involving 2 copper atoms and 2 IMP molecules, with each Cu bound to the N-7 of one molecule and phosphate of the other, producing a stacked configuration similar to that previously found for the Cu(II) complexes of 3' and 5'AMP.

In contrast to copper(II), manganese(II) ions bind to the same site, N-7, regardless of pH. Thus with IMP, as well as AMP, Mn(II) ions tend to select

a specific site that is not influenced by environmental conditions.

E. The effect of Cu(II) ions on the conformation of oligonucleotides, polynucleotides, and copolynucleotides. As we have seen, the interaction of metals with mononucleotides seems, under certain conditions, to order the nucleotides into a stacked configuration. On the other hand, some metal ions, e.g. Cu(II), unstack the stacked polynucleotide structures. In order to bridge the difference between these two systems a study of the interaction of Cu(II) with oligonucleotides (mainly oligo A's) has been undertaken. Oligo A's ranging from dimers to hexamers were studied, both without an end phosphate and with a 2',3' cyclic end phosphate. For all oligomers, except ApA, as Cu(II) is added there is an initial increase in the magnitude of the ORD, followed by a decrease at high Cu(II) concentrations. The relative concentration at which Cu(II) starts to decrease the ORD depends on the chain length and the concentration of oligomers. The decrease in ORD occurs at a lower relative concentration of Cu(II) for longer oligomers and higher concentrations of oligomers. ApA, which has only one phosphate, displays no increase in magnitude of the ORD with Cu(II), but does display only a small decrease in the ORD even at a 20-fold excess of Cu(II). The changes in the uv spectra are, as expected, smaller than the ORD changes. They generally parallel the changes in the ORD with an increase in magnitude of ORD accompanied by a decrease in the uv absorbance and vice versa.

The increases in ORD at low Cu(II) concentration are clearly attributed to an interaction with the phosphate which probably eliminates the repulsive effects between the negative phosphates. The bases are then able to assume the preferred orientation for base stacking. A similar ORD increase is observed with 0.1M NaCl. Mg(II), and spermine which also interact with the phosphates (but not the bases) enhance the stacking even more than Cu(II).

The decrease in ORD at high Cu(II) concentrations must be due to base interaction. However, the concentration of Cu(II) at which the ORD begins to decrease is much higher for the hexamer than for poly A, and higher still for the shorter oligomers. The chain length dependence could result from one Cu(II) molecule effectively unstacking a long sequence of bases or from a highly cooperative unstacking. Possibly Cu(II) bound to a given phosphate in a longer oligomer helps to neutralize charges on adjacent phosphates, thus requiring lower Cu concentration for maximum stabilization, and therefore a lower concentration of Cu can begin the destabilization.

Since uridine does not stack, a random copolynucleotide of U with another base can be considered a model for stacked oligonucleotides connected by non-interacting residues. Therefore poly (C,U) was investigated to determine whether Cu(II) affects its ORD spectrum in the concentration range found for polymers or only in the much higher concentration range found for oligomers. Poly(C,U) has some ordered structure at neutral pH which on the basis of a broad melting is very similar to single stranded segments of C's. Furthermore, no double stranded structure seems to be formed in acid pH, suggesting that these stacked segments are actually quite short, (double stranded structures of C are observed for chain lengths of 6 or greater). Despite this oligomeric character, Cu(II) produces a decrease in the ORD in the same concentration range as for poly C. These results suggest that the ability of

Cu(II) to unstack poly C does not depend on having long sequences of C's but on having the stacked C's as part of a long polymer.

F. Further studies on the reversible denaturation of polynucleotides by metal ions. It is becoming increasingly clear that runs of identical nucleotides are of significance in RNA molecules; the study of homopolynucleotides therefore gains importance. Previously the ability of metals to stabilize various conformations of polyribonucleotides has been established, but poly(G), which is most difficult to characterize, has been least well understood. Solutions of poly(G) are stable towards heat and acid, yet Cu(II), Cd(II) and Ni(II) destabilize the double helical structure, considerably lowering T_m. In the absence of these metal ions denatured poly(G) cannot be completely renatured, but denaturation in their presence can be completely reversed by EDTA. Possibly these metal ions can form crosslinks between G pairs in poly(G) in the same manner as has been postulated with DNA. Zn(II) and Mg(II) do not produce reversibility of the denaturation.

When DNA is denatured in the absence of divalent metal ion, the AT-rich regions are melted before the GC-rich regions, because of the greater stability of the latter, as determined by differences in T_m at 260nm (specific for AT) and 230nm (specific for GC). In the presence of divalent metals [e.g. Zn(II)] there is no difference in the susceptibility of various regions of DNA to denaturation. The metal ions bring about a much more cooperative denaturation process.

G. Nuclear magnetic resonance studies on the binding of copper(II) and manganese(II) to RNA. Much work has been previously reported to establish the binding sites of metal ions on mononucleotides and homopolynucleotides. This work has now progressed to the much more difficult study of metal binding to heteropolymers, e.g. ribosomal RNA. The peaks in the RNA spectrum were assigned by comparison with previously observed peaks in the spectra of homopolymers. Reasonably good resolution was attained in a 80% DMSO-d_6-20% D_2O solvent, in which the RNA is destacked. Confirmation of peak assignments was made by deuteration of RNA at elevated temperature, using the fact that the H-8 peaks of G and A deuterate at different rates and the H-6 peaks of C and U are not readily deuterated.

Studies in DMSO/D_2O mixtures as well as studies in water indicate that Cu(II) binds much more strongly to the bases in DNA than does Mn(II). Both metal ions broaden H-8 of G preferentially, than H-8 and H-2 of A, H-5 and H-6 of C and U and the ribose H-1' are broadened at much higher metal ion concentrations. Thus the binding affinity for both metal ions in RNA is G > A > C,U.

An interesting effect occurs when either Cu(II) or Mn(II) have broadened the G and A peaks. At this metal concentration the H-5 of U and C, and the H-1' of ribose areas are sharpened, and only higher metal concentrations will again broaden these peaks. The sharpening is attributed to an unstacking of the bases.

The assignment of NMR peaks in a heteropolymer is complicated by the fact that the peaks of the bases appear to shift when other bases are adjacent to them.

Although this phenomenon causes difficulty, it may prove to be an extremely useful tool in determining the effect of one base on another in a heteropolynucleotide structure.

Significance to Bio-medical Research and the Program of the Institute. The ability of metal ions to control the conformation of polynucleotides signifies that metal ions in biological systems can determine whether or not the polynucleotide is in a favorable conformation for its genetic activity. It is hoped that the studies on the reaction of antitumor platinum complexes with DNA will not only elucidate the pharmacological process but will also demonstrate the mechanism of the inhibition of DNA replication by metal ions. The ability of metal ions to stack mononucleotides in a manner reminiscent of the stacking in RNA and DNA has important implications to the participation of metal ions in the synthesis of these molecules. The determinations of the binding sites of metal ions on RNA is important, since metal ions are required in the stabilization of RNA, and at the same time can deform it in such a way as to inactivate it.

Proposed Course of Project: It is intended to determine the concentration of metal ions in biological materials, and the changes in these concentrations with age, as well as to determine changes in the permeability of cells to metal ions or metals complexed to small and large molecules, as a function of age. It is intended to continue the studies on the localization of metal ions on polynucleotides, and to determine the effects of metal binding on the chemical and biological properties of these substances. It is hoped to make use of inorganic reagents tailor-made for interaction with groupings on nucleic acid molecules.

Honors and Awards: None

Publications:

Berger, N. A. and Eichhorn, G. L., "Interaction of Metal Ions with Polynucleotides and Related Compounds. XVIII. The Multiplicity of Reactions of Copper(II) with Inosine and Its Derivatives", J. Amer. Chem. Soc., 93, 7062 (1971).

Shin, Y. A., Heim, J. M. and Eichhorn, G. L., "Interaction of Metal Ions with Polynucleotides and Related Compounds. XX. Control of the Conformation of Polyriboadenylic Acid by Divalent Metal Ions", Bioinorganic Chemistry 1, 149 (1972).

Eichhorn, G. L. and Berger, N. A., "The Interaction of Copper(II) Ions with Purine Nucleosides and Nucleotides", Jerusalem Symposia on Quantum Chemistry and Biochemistry IV, The Purines, Theory and Experiment, ed. by B. Pullman and E. D. Bergmann, 1972.

Serial No. HD-GMA2
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Degradation of Nucleic Acids by Metal Ions

Previous Serial Number: Same

Principal Investigators: J. Butzow (100% time)
G. Eichhorn (15% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	1.65
Professional:	1.15
Others:	.50

Project Description:

Objectives: (1) To render metal ion degradation of polynucleotides useful in the detection of nucleotide sequence and in selective degradation of ribonucleic acids. (2) To study the mechanism of the metal degradation reaction. (3) To utilize the ability of metal ions to degrade polynucleotides in the probe of the structure of biological macromolecules such as 5sRNA.

Methods Employed: Details of the influence of nucleotide sequence in ribonucleic acids in the zinc cleavage reaction and of the mechanism of the reaction are followed by means of kinetic measurements of degradation and determination of the degradation products, using trimers of known primary structure as substrates. To determine the positions of preferential cleavage of the 5sRNA molecule, the latter is reacted with zinc(II) for a limited amount of time. The 5'hydroxyls created by this cleavage are labelled by esterification with ^{32}P-orthophosphate by means of polynucleotide kinase transfer from ATP. The labelled polynucleotide fragments are then cleaved further by T1-RNAse digestion at the 3' side of the guanylate residue nearest the zinc cleavage site. The oligonucleotides so formed are separated with respect to chainlength. The nucleotide residues at the 5' end of each chain length class are then determined as labelled 5'mononucleotides generated by complete digestion with venom phosphodiesterase.

Major Findings: A. Studies of metal ion induced cleavage of 5sRNA. Our previous general study on nucleotide specificity of zinc cleavage of heterogeneous RNA indicated greatest specificity at high zinc/nucleotide residue ratios. Cleavage of 5sRNA was carried out using 10 moles Zn/nucleotide residue, pH 7, at 40°, a temperature at which cleavage was expected to proceed slowly but not too slowly, and at which the initial RNA structure would not be significantly altered from its native configuration as determined by ORD experiments. On the average about one phosphodiester bond per molecule (120 residues) was cleaved in 10h. The initial rates were considerably faster than this, the first bond being cleaved in 4 to 6 h. There also appeared to be an interim levelling off at about 2 bonds, when the reaction was followed further; this could indicate that the structure of the RNA has two easily cleavable bonds up to this point, after which a structural change occurs.

The pattern of zinc cleavage within the 5sRNA chain was determined, for a cleavage level of 1 bond per chain, through phosphorylation (^{32}P label) of the 5' hydroxyls created. The different portions of the chain involved in the cleavage were delineated by observing the chainlength of the labelled fragments produced by specific enzymatic cleavage at the guanylate residues, and the 5' residues in each such chainlength class. That the RNA molecule has been cleaved and also labelled at several places is clear from the fact that label was found for several chainlength classes. Analysis of the labelled 5' nucleotide results reveals that the distribution in each chainlength class is far from random. Considering published models for the structure of native 5sRNA, the results are accounted for by cleavages occurring mostly in stretches of the chain which are not base paired. In view of the variety of end groups detected after an average of only one Zn cleavage per chain, it is clear that there has been some randomization; we suspect this may be due to nuclease contamination in the polynucleotide kinase preparation.

B. Formation and breakdown of poly(rA)-poly(rU) complexes, and cleavage, in the presence of Zn or Mg ion. As part of an investigation of cleavage in ribopolymer and ribopolymer-deoxypolymer hybrid complexes, the formation and breakdown of poly(rA)-poly(rU) complexes in the presence of Zn ion was examined. In the neighborhood of pH 7, polymer phosphate equivalent concentrations of Mg ion or high concentrations of Na ion stabilize the formation of base paired helical complexes of poly(rA) with poly(rU), a double stranded complex A:U or a triple stranded complex A:2U. When the mole fraction of U is ½, A:U is exclusively formed; when the mole fraction of U is 2/3, A:2U is exclusively formed. These two reactions can be followed separately at wavelengths at which only one or the other reaction is detected. Thus, at 284nm, $A+U \rightleftarrows A:U$ is detected, while at 280nm, $A+2U \rightleftarrows A:2U$ is detected. Zinc ions dramatically change these equilibria. In the presence of zinc (2 Zn/nucleoside residue), at 284nm no change in absorbance occurs at a mole fraction of U exceeding 2/3, while at 280nm there is a continuous decrease in absorbance all the way from mole fraction U of 0 to $\sim$ 2/3, indicating the formation of A:2U. A:2U can be formed intermediate to A:U. Thus Zn ions favor the formation of A:2U at all proportions of A and U. This is also illustrated in melting experiments at mole fraction U of ½ or of 2/3, as followed by the 280nm absorbance.

On heating to higher temperatures, A:2U breaks down into single strands, giving an increase in the 280nm absorbance. With Mg this increase is sharpest around 75°; with Zn, around 65° (2 Mg or 2 Zn/nucleotide residue). The lowering of the melting temperature for the A:2U breakdown in the presence of Zn is analogous to what occurs in the melting of DNA, and is presumably related to the fact that zinc binds bases more strongly than Mg. With Zn, as the temperature is increased beyond 70°, the 280nm absorbance rapidly decreases; with Mg, there is only a small decrease. If the temperature is lowered after reaching about half the maximum absorbance increase, the absorbance decreases, and on reheating (allowing 24h for reformation of complex) the transition for breakdown of A:2U reoccurs at the same temperature. If, however, the temperature is raised to 90°, no transition occurs on cooling and reheating. This suggests that rapid cleavage of the liberated single strands is brought about by Zn or Mg at the high temperatures. Single stranded polynucleotides should be very extensively cleaved by Zn ion, according to our previous reports, during the time in which the temperature is being raised beyond the 65° transition.

The absorbance changes at 280nm on heating poly(rA)-poly(rU) in the presence of Zn provide a graphic example of the consecutive or simultaneous involvement of a metal ion in governing polymer conformation and in producing polymer degradation, exemplifying various ways in which a metal ion can interact with a nucleic acid.

C. Nucleotide specificity in the Zn cleavage of oligoribonucleotides: GpUpU. Since the previously reported study on the zinc cleavage of triribonucleoside diphosphates at their two phosphodiester bonds was carried out, an additional heterotrimer, GpUpU, which can be related to UpUpG and to UpUpU, has become available. Accordingly, we have conducted a rate study of its cleavage by Zn ion under the conditions used previously (2 Zn/nucleoside residue, 64°, pH 7).

GpUpU displays cleavage kinetics similar to those of UpUpU, and not UpUpG; thus, the overall cleavage rate and the rate of cleavage at the phosphodiester bond nearer the 5' end (and the preference for cleavage at that bond) are quite close to the respective parameters for UpUpU. UpUpG, on the other hand, was previously found to be cleaved much more rapidly than UpUpU, the bond nearer the 5' end itself being cleaved at a much higher rate, giving a much greater preference for cleavage at that bond.

The findings with GpUpU re-emphasize the sequence dependence of the cleavage reaction. GpUpU is expected to have little or no base stacking, as also are UpUpU and UpUpG. The comparisons in the kinetics of zinc cleavage among UpUpU, UpUpG, and GpUpU, then indicate an effect of another type of interaction involving the G base, in which the position of G in the trimer is decisive.

Significance to Bio-medical Research and the Program of the Institute. 5sRNA is a component of the 50s subunit of E. coli ribosome. It is the simplest ribosomal component and the one most readily capable of structural elucidation. In order to understand protein synthesis, the structure of the ribosome must be understood; the studies with zinc are designed to aid in

this undertaking. The conditions under which polynucleotides form double strands or triple strands are of great importance in the replication of DNA and the transcription of RNA, since both processes involve the formation of a new polynucleotide strand on a DNA duplex template.

Proposed Course of Project: Because of the difficulty of obtaining polynucleotide kinase completely free of ribonuclease activity, the 5sRNA degradation will be studied using alternative techniques. One such technique is the utilization of 5sRNA prelabelled in the base moiety.

Honors and Awards: None

Publications: None

Serial No. HD-GMA3
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Structure of Nucleoproteins

Previous Serial Number: Same

Principal Investigators: G. Eichhorn (15% time)
Y. Shin (70% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	1.05
Professional:	.85
Others:	.20

Project Description:

Objectives: (1) To determine the conditions for the binding of proteins to nucleic acids, (2) to examine the mediation of this binding by metal ions, (3) to elucidate the structure of chromatin with the help of these model systems, (4) to understand how cellular differentiation and aging may be related to nucleoprotein structure.

Methods Employed: The interactions of proteins and nucleic acids, and the effect of metal ions on these interactions are studied by optical rotatory dispersion and circular dichroism techniques, to determine conformational changes, and by infrared and nmr techniques, to determine binding sites. Models containing selected characteristics of chromatin are examined to determine which characteristics are responsible for chromatin function. Polylysine is specifically studied to understand the function of lysine-rich histone.

Major Findings. A. The critical nature of electrolyte concentration in determining the formation or destruction of DNA-polylysine complex. High salt concentrations dissociate the complex between DNA and polylysine by providing a positive environment for the DNA in which the positively charged lysines cannot bind the negative charges on the phosphate. Below a critical electrolyte concentration, however, electrolyte stabilizes the DNA-polylysine interaction. Examination of ORD curves of DNA-polylysine

solutions at varying salt concentrations reveals that successive increments of salt continuously convert unassociated DNA to the DNA-polylysine complex, until the electrolyte (NaCl) concentration is 0.9M. All ORD spectra are related by isosbestic points, demonstrating that only one complex is produced, with no intermediates. NaCl concentrations higher than 0.9 diminish the rotatory intensity of the solution, as the complex is dissociated. Divalent metal ions stabilize the DNA-polylysine at concentrations many orders of magnitude below univalent ions; thus 10^{-4}M Ni(II) is as effective as 0.6M NaCl.

B. DNA irreversibly bound to DNA-polylysine. It had previously been observed that DNA-polylysine (1:0.5) prepared by dialysis from high salt, and centrifugation to separate uncomplexed DNA, exhibits a two-step melting curve, the first step having a T_m of uncomplexed DNA and the second step corresponding to the DNA-polylysine complex. The trivial explanation of this phenomenon was that uncomplexed DNA has not been completely removed from the complex. However, the DNA-polylysine complex can be repeatedly repelleted and redissolved; after the first pelleting, no DNA remains in the supernatant, and each successive pellet retains the identical proportion of material with the two different melting points. The two-step melting curve must therefore be an intrinsic characteristic of the DNA-polylysine complex. Conceivably free DNA is irreversibly bound to the complex. The possibility exists that polylysine winds around a protein of the DNA duplex in such a manner as to stabilize that portion (high T_m) while keeping another portion of the DNA in a condition susceptible to unwinding (low T_m). Such a structure is characteristic of the model of chromatin proposed by F. H-C. Crick to account for genetic regulation.

C. Nature of copper complex of DNA-polylysine. It had been previously observed that when a 1:0.5 solution of DNA-polylysine in the presence of an equimolar (with DNA) quantity of copper(II) is heated to 62°, it exhibits an ORD curve very different from that of DNA-polylysine in the absence of divalent metal; in fact the ORD curve is practically a mirror image of the usual DNA-polylysine curve. This complex has now been thoroughly investigated. It is an extremely stable substance, and has been maintained for long periods of time at a variety of temperatures. The complex requires the 2:1 ratio of DNA to polylysine; it does not form in the presence of lower or higher quantities of polylysine. Whereas DNA-polylysine solutions containing excess DNA can generally be separated into complex and free DNA, no such separation is possible for the complex with copper. It thus appears that exactly one mole of "free" DNA is intimately associated with the DNA-polylysine complex. This "free" DNA does not function merely to solubilize DNA-polylysine by supplying excess charge; this has been established by attempting to substitute for the "free" DNA such polyanions as polyglutamic acid, poly d(AT), and poly(U), with negative results. In fact, DNA itself added to a DNA-polylysine pellet in the presence of copper(II) does not produce this unusual substance, indicating that the free DNA must be present during the formation of the complex. The copper(II) as well as the excess DNA, must be present from the beginning. No other metals can substitute for copper, and other polycations that have been tried do not substitute for polylysine. The structure of this complex is unknown, but it can be speculated that it

contains copper forming bonds between the DNA-polylysine complex and an extra molecule of DNA.

D. The reaction of polynucleotides with small polycations. It is now widely believed that the histones, polylysine, etc. protect the DNA structure by tying the strands together through phosphate binding to both strands. When bound to the polypeptides, the conformation of the DNA is altered. It is of some interest to determine what are the minimum requirements for an organic polycation to alter the conformation of a polynucleotide. Poly A at pH 7 (single helical) was allowed to react with ethylenediamine, spermidine, and spermine, which contain 2, 3, and 4 positive sites respectively, and also with polylysine which has many such sites. Ethylenediamine had no effect, but all the other polycations brought about the randomization of the poly(A) structure. It is therefore assumed that 3 or more positive sites simultaneously binding to phosphates are required to untwist the poly(A) structure. The structure of poly(C) is not affected by any of these cations, presumably because the glycosidic bond is stiffer and thus less readily distorted by them. All of the cations are capable of organizing poly(U) into a double helix. In this respect they function in a manner analogous to histone with DNA.

Significance to Bio-medical Research and the Program of the Institute: The ability of metal ions to secure the binding of protein to DNA at one concentration and then to dissociate the complex at another concentration can be an important regulatory mechanism for the binding and dissociation of proteins from DNA during cell differentiation and aging. Metal ions binding free DNA to nucleohistone as in the copper complex studied here would produce aggregates that could inhibit RNA synthesis. The delicate equilibria between metal ions, polylysine and DNA suggest mechanisms by which many phases of chromatin activity can be controlled.

Proposed Course of Project: It is intended to study the nature of the binding of DNA and polylysine, and to compare polylysine model systems to polyarginine model systems. These models could then be used to understand the structure of chromatin.

Honors and Awards: None

Publications: None

Serial No. HD-GMA5
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Polymers as Biological Reagents

Previous Serial Number: Same

Principal Investigator: J. Pitha (80% time)

Other Investigators: P. M. Pitha
R. Adams
N. Nelson
E. Borden

Cooperating Units: Baltimore City Hospitals
Johns Hopkins University

Man Years:

Total:	1.80
Professional:	.80
Others:	1.00

Project Description:

Objectives: This program is an expansion of the previous program "Analogs of Nucleic Acids". Working with the polymeric analogs we found that some of them have unexpected biological potency and the present program tries to exploit these findings. The objective of the program is to prepare macromolecules which would easily be taken up by cells and would have a high escape rate from pinocytotic vesicles so that a large portion of the macromolecules will penetrate into the cytoplasm. The macromolecules will furthermore have one of the following properties. (1) Carry groups known to be parts of biological macromolecules (e.g. uracil residues). (2) Be complexed with biological macromolecules (e.g. complex with polynucleotides). (3) Will be hydrolyzed into small, biologically active fragments.

Methods Employed: Both chemical and biological methods must be used in the project. Macromolecules are synthesized using chemical reactions and their biological potencies tested on human cells grown in vitro. The simple uptake of macromolecules by cells is studied at the Gerontology Research Center; biological effects, especially the antiviral protection, is done in collaboration with Dr. P. M. Pitha on the premises, with the support of the Department of Medicine, Johns Hopkins School of Medicine.

Major Findings: A. Vinyl analogs of nucleic acids. In a collaborative study with Drs. Pitha (Hopkins), Grunberger and Hoyer (Columbia) we found that vinyl analogs of polynucleotides strongly inhibit the translation of polynucleotide templates into proteins. To clarify the mechanism of inhibition and elaborate on the finding, the following aspects were studied. a) A copolymer system containing uracil and adenine residues in a polyvinyl polymer was developed. Vinyl copolymers with varying A/U ratio were prepared and found in general to be less reactive toward the complementary polynucleotides than the vinyl homopolymers. b) The position of equilibrium was studied in systems containing an analog complex with the complementary polynucleotide and the polynucleotide corresponding to the analog. The study revealed higher stability for polynucleotide-polynucleotide complexes than for the complexes of the type polynucleotide-analog.

B. Preparation of carriers for nucleic acids. Ribonucleic acids are known to be taken up by mammalian cells only sparingly; the uptake can be potentiated by polybases, e.g. poly-L-lysine or DEAE dextran. We based our work in the design of new carriers on the assumption that the high molecular weight aggregates of nucleic acids, especially when they have lower charge to mass ratio, will be taken up more easily. In the preparation of such aggregates we used two types of processes: 1) A physical approach, which does not involve the formation of any new bonds. The following systems were tested: a) nucleic acids adsorbed on mica, b) nucleic acids adsorbed to carbon particles, c) nucleic acid aggregates formed by precipitation by polyethylene glycol, d) nucleic acid aggregates formed by precipitation by polyvinylpyrrolidone. The mica system proved to be the most promising one and is presently being developed into a more general method for potentiation of effects of nucleic acids on cells grown in tissue culture. 2) Chemical approaches, in these, new chemical bonds between polynucleotide and the carrier are produced. The following carriers and methods were used: a) Sepharose beads activated by the CNBr procedure. This method gave a product of relative low potency but is convenient from the preparative point of view. During our work with this system it came to our notice that a larger research team in the pharmaceutical industry (Merck) was following the same path and therefore we probably will abandon our efforts in this direction. b) Dextran macromolecules activated by the CNBr procedure: This method was found to yield only mixtures which are difficult to separate, c) the formation of a polynucleotide-cellulose bond by ultraviolet light: Active preparations are made very easily; in future work the proper sizing of cellulose particles will have to be investigated, d) the formation of a polynucleotide-dextran bond by ultraviolet light; by careful modification good yields of complex were obtained.

C. Uptake of polymers by cells. The dependence of uptake on the structure and molecular weight of macromolecules has been investigated. As a first step a series of radioactive polysaccharide macromolecules has been prepared from commercially fractionated Dextran. The average molecular weights of the fractions are 10^4, $2x10^4$, $1.1x10^5$, $5x10^5$ and $2x10^{+6}$ and in future work the uptake of these macromolecules by cells will be measured, and as these molecules are only negligibly degraded in mammalian cells, the whole cycle of phagocytosis may be investigated by autoradiographic methods.

D. Age dependence of effects of polymers on cells. The amount of cellular

uptake of macromolecules from the medium may be expected to depend on the age of the cells. In order to study this aging phenomenon, diploid human cells of different passage level have been maintained in the laboratory and the age dependence of the uptake of polystyrene beads has been measured. The present data show considerable scatter and for the final quantitative evaluation more work is necessary.

Furthermore, in co-work with the Oncology Unit of Hopkins University the dependence of cellular level on the age and passage level of human fibroblast cells is being studied. We concentrated on the age dependences of two parameters: a) The amount of the total response to induction of an antiviral defense, b) kinetics of response after the pulse induction.

The present results show that the antiviral defenses are induced most effectively in the middle of the lifespan and continue to be well inducible up to around 90% of the total in vitro lifespan. The doses necessary for the maximal protection at first decreases with increasing age and then increases; the minimum being around passage 30-35.

At the Oncology Unit in the project are involved: P. M. Pitha, R. Adams, N. Nelson, and E. Borden.

Significance to Bio-medical Research and the Program of the Institute. Modification of the cells by the compounds which enter the cytoplasm by pinocytosis has been used only sparingly. With the proper chemical design of macromolecules this may prove to be a very effective way, as in contrast to the transport of small molecules, pinocytosis is relatively non-specific and a large variety of compounds can be brought into the cytoplasm.

As related to the study of aging, the present project can yield some information on the ability of cells to regulate their protein synthesis and on the integrity of the proteins synthesized. These two questions have crucial importance for the study of the molecular mechanism of aging. The advantage of using polymers for this study lies in the circumstance that this response does not disappear during the prolonged in vitro growth of the cells.

Proposed Course of the Project: The present results show that pharmacologically effective polymers and carriers of nucleic acids can be prepared. Furthermore, the macromolecules can be used on in vitro cells in the same way as physiologists use physical or horomonal stimuli, and the competence of in vitro aged cells can be quantitated. To pursue the project successfully the present chemical work and and methods will have to be supplemented by better cell culture techniques. This improvement is especially crucial for the study of aging aspects, as the preparation of healthy populations of in vitro aged cells which react in a uniform way is technically difficult.

Honors and Awards:
J. Pitha was asked to present a talk at a symposium, "Conformation of Nucleosides and Polynucleotides" at the First European Biophysics Congress, Baden, Austria, September, 1971.

J. Pitha was invited to present a talk at the American Chemical Society

Symposium, "Polymers as Chemical Reagents", New York, September, 1972.

Publications:
Pitha, J., Pitha, P. M. and Stuart, E., "Vinyl Analogs of Polynucleotides", Biochemistry 10, 4595 (1971).

Serial No. HD-GMA6(1)
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mispairing of Nucleotide Bases

Previous Serial Number: Same

Principal Investigators: G. Eichhorn (20% time)
J. Pitha (20 % time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	.90
Professional:	.40
Others:	.50

Project Description:

Objectives: (1) To determine the conditions under which mispairing of the complementary bases occurs between polynucleotide strands in order (2) to understand the ways in which misreading of the genetic code can occur in order (3) to understand how errors that could lead to the aging of organisms can occur in the apparatus for the transmission of genetic information.

Methods Employed: It is known that metal ions of the wrong type or in the wrong concentration can bring about the misreading of the genetic code in protein synthesis, resulting in the production of proteins containing incorrect amino acid sequences. Such errors can arise through mispairing of bases in codon-anticodon recognition. The following hypothesis is devised to account for mispairing. It is known that high concentrations of divalent ions stabilize the interaction of polynucleotide strands. In the absence of the divalent metal ions these interactions are therefore relatively unstable and require base complementarity; in the presence of these ions the interactions are stabilized to such an extent that sensitivity to complementarity is lost, and mispairing then occurs.

This hypothesis can be tested by studying the binding of homopolynucleotides to heteropolynucleotides containing one base that is complementary and one base that is not complementary to the base in the homopolymer. The extent of mispairing is measured by (a) stoichiometric studies of the reaction between the two polynucleotides and (b) by nuclear magnetic resonance

studies that detect only non-hydrogen bonded bases.

Major Findings: A. Stoichiometry studies. The binding of poly(I) to poly (A,C) and to poly(U,C) was studied, using the method of continuous variation, in the presence of various kinds and concentrations of metal ions. Sodium ion at 0.1M concentration causes a reaction of 2 moles (nucleotide) of either poly(A,C) or poly(U,C) with 1 mole poly(I). This reaction ratio is as expected for a completely complementary reaction, in which I residues react only with C residues, and do not react with either A or U residues. The addition of divalent metal ions at much lower concentration (10^{-2}M), on the other hand, leads to the reaction of only 1 mole of either poly(A,C) or poly (U,C) with 1 mole of poly(I), indicating that not only C residues, but also A and U residues react with I residues under these conditions. The nature of the divalent ions does not seem to matter, since Mg(II), Mn(II) or Ni(II) produce the same results. Thus it appears that divalent ions are able to induce mispairing in these systems.

When increments of Mg(II) are added to a mixture of poly(U,C) and poly(I), there is a transition, at approximately 10^{-2}M Mg(II), from a 2:1 reaction to a 1:1 reaction; i.e., low Mg(II) produces conditions sensitive to complementarity, while high Mg(II) promotes conditions under which mispairing occurs.

B. NMR studies to confirm the mispairing phenomenon. The NMR lines characteristic of a nucleotide base are broadened when that base is engaged in hydrogen bonding. Evidence that divalent metal ions induce mispairing was obtained from the fact that base peaks, which could be observed in mixtures of poly(A,U) and poly(I) in the presence of sodium, were broadened upon the addition of divalent metal ions.

Significance to Bio-medical Research and the Program of the Institute: The accumulation of errors that would be produced during protein synthesis by mispairing of nucleotides of the kind being studied would lead to deleterious effects in an organism, according to one of the more popular aging theories. Thus the study constitutes a chemical model for a theory of aging.

Proposed Course of Project: We intend to investigate other polynucleotide systems, and thus to determine whether mispairing can be induced by metal ions in the binding of transfer RNA to messenger RNA.

Honors and Awards: None

Publications: None

Serial No. HD-AG17(8)(c)
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Relation of Structure and Function in Hemoglobin

Previous Serial Number: Same

Principal Investigator: J. Rifkind (60% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	.80
Professional:	.60
Others:	.20

Project Description:

Objectives: (1) To study the binding of ligands to hemoglobin. (2) To determine the role of the protein in controlling the binding of ligands to hemoglobin. (3) To study the mechanisms for maintaining hemoglobin in its functional form.

Methods Employed: The autoxidation of hemoglobin is investigated under various conditions in the presence of various complexing agents and metal ions. Oxygen binding curves are analyzed in order to determine the binding constants and free energies of interaction involved at each step of the oxygenation. These studies help to elucidate the mechanisms for the efficient transport of oxygen.

Major Findings: A. The autoxidation of hemoglobin. Oxygen does not bind to oxidized hemoglobin. Therefore, autoxidation decreases the oxygen binding capacity of hemoglobin. Our previous studies, on the stabilizing effect of strong metal complexing agents and the destabilizing effect of certain metal ions, suggested the involvement of metal ions in the autoxidation of hemoglobin.

We have now compared the effect of the very strong complexing agents, ethylenediaminetetraacetic acid (EDTA) and triethylenetetramine, with ethylenediamine, a much weaker complexing agent. As expected, ethylenediamine also prevents autoxidation, but only at a concentration which is more than two orders of magnitude higher than that required for the same stabilization by

EDTA and triethylenetetramine.

We have also been able to demonstrate that the metal ion most likely responsible for the autoxidation is Cu(II). Many other metal ions, including those which are stronger oxidizing agents than Cu(II), do not produce any significant autoxidation even at a concentration of $1x10^{-4}M$. However, $1x10^{-7}M$ Cu(II) very significantly enhances the rate of autoxidation. Because of this drastic effect, the presence of less than $1x10^{-7}M$ Cu(II) can explain the rate of autoxidation found with our hemoglobin preparations.

Studies on the addition of Cu(II) to deoxyhemoglobin indicate that Cu(II) can also oxidize hemoglobin in the absence of oxygen. However, the effect is not catalytic and stoichiometric concentrations of Cu(II) are required. Therefore, oxygen must be involved in regenerating the divalent Cu(II) from the monovalent Cu(I) which is formed when Cu(II) oxidizes hemoglobin.

In order to evaluate the importance of the complexing agents in preventing autoxidation, we have investigated the effect of EDTA on the rate of autoxidation under various conditions.

One of the most serious problems involved in studying the oxygenation of hemoglobin is the surprisingly rapid rate of autoxidation at low oxygen pressures when hemoglobin is only partially oxygenated. EDTA was also found to lower the rate of autoxidation under these conditions.

Nonpurified hemolyzed cells are autoxidized much less readily than purified hemoglobin. Nevertheless, we find that EDTA further lowers the rate of autoxidation. This behavior suggests that complexing agents can possibly contribute to the stabilization of hemoglobin in the erythrocyte.

The stabilizing effect of EDTA was also found to be insensitive to the ionic strength and hemoglobin concentration, even though these parameters do influence the rate of autoxidation.

B. The interaction energy responsible for cooperative oxygenation. The physiological function of hemoglobin requires cooperative binding of oxygen in order to facilitate an efficient uptake and release of oxygen. Therefore, the oxygenation of one hemoglobin subunit influences the oxygen affinity of the other subunits. The free energy of interaction responsible for the cooperative binding has previously been obtained from the Hill plot.

We have shown that the interaction energy obtained in this manner is determined exclusively by the affinity for binding the first and last oxygen molecules. A more valid interaction energy can be obtained from the four stepwise binding constants. This method furnishes information regarding the interaction energy at each step of the oxygenation. An analysis of the interaction energies determined from binding data for human, horse and sheep hemoglobin at pH 7 and pH 9 suggests that the detailed mechanism for cooperative oxygenation depends on both the pH and the species.

Significance to Bio-medical Research and to the Program of the Institue: The

physiological role of hemoglobin is to transport oxygen from the lungs to the cells. The efficient uptake and release of oxygen requires cooperative oxygen binding and the proper regulation of the oxygen affinity. A study of the mechanism for cooperative oxygenation thus elucidates a vital function of organisms. A mechanism for inhibiting the autoxidation is crucial for the proper functioning of hemoglobin. Autoxidation, no only decreases the oxygen binding capacity, but also decreases the cooperativity and increases the oxygen affinity. The oxidation of hemoglobin seems to be at least partially responsible for the aging of the erythrocyte. The possibility also exists that a breakdown in the erythrocyte mechanism for maintaining functional hemoglobin in the aging organism can produce changes in the ability of the organism to transport oxygen from the lungs to the cells.

Proposed Course of Project: (1) We plan to study the mechanisms involved in the autoxidation of hemoglobin. A method to effectively inhibit the autoxidation in purified systems will be developed. (2) The influence of various small molecules, such as complexing agents and metal ions, on the functioning of hemoglobin will be studied. (3) We intend to study the oxygenation of hemoglobin in order to obtain further information regarding the interaction between hemoglobin subunits. (4) We would like to determine if there is a change in the ability of erythrocytes to transport oxygen with age. An attempt to explain the origin of any differences will be undertaken.

Honors and Awards: None

Publications:
Rifkind, J. M., "Hemoglobin and Myoglobin", in Inorganic Biochemistry, Eichhorn, G. L. (Editor), Elsevier, Amsterdam, in press.

Serial No. HD-AG30
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Molecular Chemistry Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Function of Metal Ions in Enzymatic Reactions

Previous Serial Number: Same

Principal Investigators: P. Clark (90% time)
G. Eichhorn (15% time)

Other Investigators: None

Cooperating Units: Baltimore City Hospitals

Man Years:

Total:	1.05
Professional:	1.05
Others:	0

Project Description:

Objectives: (1) To convert non-specific nuclease enzymes into base-specific cleavage reagents useful in the determination of nucleotide sequence. (2) To elucidate the mechanism by which metal ions activate or inhibit enzymatic reactions.

Methods Employed: DNAse is an enzyme that cleaves the internucleotide bonds of DNA rather non-specifically. Since the enzyme presumably recognizes a nucleotide unit in the DNA structure, the reaction of any nucleotide base with a metal ion should prevent reaction of the enzyme with that nucleotide, so that preference would be exhibited for nucleotide sites that do bind the metals. Since different metal ions react preferentially with different sites on the DNA, it is expected that participation of a variety of metal ions in the DNAse reactions will block cleavage at different locations, thus changing the specificity of the enzyme in a variety of ways. The metal-DNA complexes are reacted with the enzyme, the resulting oligonucleotides are treated with phosphatase to remove terminal phosphate, and then with venom phosphodiesterase to hydrolyze to nucleotides and nucleosides. Analysis of the nucleosides indicates points of cleavage of DNA by DNAse.

The mechanism of the DNAse reaction is examined by analysis of the components of the oligonucleotides obtained in the enzymatic reaction, leading

to a differentiation between single-hit and double-hit mechanisms.

Major Findings: A. Increasing the specificity of the DNAse reaction by suitable metal ions. Work previously reported in which Cu(II) and Mg(II) were used to activate DNAse I in the hydrolysis of DNA showed that while the Mg(II) activation produced more or less random cleavage, the Cu(II) activated enzyme preferentially hydrolyzed phosphodiester bonds adjacent to the thymidine 5'PO4 and tended to skip the guanosine 5'PO4. These results suggested that perhaps the enzyme specificity could be modified in other ways by other metals which complex strongly with the DNA bases. Mg(II) is such a metal; Hg(II) prefers DNA with a high AT content, by specifically binding to the T residues.

Experimentally this is a difficult task, but it appears worthwhile in view of the lack of available techniques for sequencing DNA. The reaction with Hg(II) must be carried out in the presence of Mg(II) as an activator; it differs in this regard from the reaction with copper. The modified DNAse reaction is stopped with $HClO_4$ after 5% of DNA bonds have been broken (based on previously reported kinetic studies). The large fragments and unreacted DNA are precipitated along with the inactivated enzyme, leaving the soluble oligonucleotides in the acid supernatant. Care must be taken not to destroy the purines during the acid treatment. After neutralization the buffers and metal ions must be removed by desalting on a DEAE cellulose column, which adsorbs oligonucleotides more strongly than salts. This technique does not, however, removed Hg(II), since these metals are strongly complexed by the oligonucleotides. Many methods were tried to remove these metals; the technique that proved to be successful was extraction with dithizone in CCl_4. Thus finally an uncontaminated product of oligonucleotide fragments is produced. Then fragments are subjected to hydrolysis with phosphomonoesterase to remove terminal phosphates. This enzyme must be completely inactivated before the succeeding reaction with venom phosphodiesterase can be carried out. The latter enzyme is very unstable; once it has acted, the cleavage products must be rapidly removed before the phosphomonoesterase is reactivated and dephosphorylates nucleotides that are not at cleavage points of the DNAse. The procedure requires several weeks and must be carried out under sterile conditions.

In the hydrolysis of Hg-DNA, the analysis of nucleosides, which determines the sites of cleavage, yields the following results: A: 52%, g: 21%, C: 10%, T: 16%. The reaction when activated by magnesium yields, for A, G, C, and T, 19, 24, 17, and 40% respectively. Thus there is preferential cleavage at A and a greatly decreased rate of cleavage at T. The latter result is precisely what would be expected from the preferential binding of Hg(II) to T, which would cause the DNAse to fail to recognize the T sites and thus to ignore them. The reaction with copper produces a decreased cleavage rate at G, the preferred binding site of copper, and a greatly increased rate at T. Thus the effects of copper and mercury on the DNAse reaction are in line with the hypothesis which led to these studies; i.e., that reaction is inhibited next to the bases that bind most strongly to the metal ions. It would appear that the metal modified DNAse reaction will be helpful in sequencing DNA.

B. Does the metal-modified DNAse reaction cause cleavage on one chain at a time, or are both chains cleaved simultaneously? It has been established that the Mg(II) activated reaction of DNAse cleaves one chain at a time, whereas experiments in the literature have been interpreted as indicating that the Mn(II) activated enzyme cleaves both chains simultaneously. An ingenious technique has been developed for determining which of these two mechanisms operates. Whereas the determination of nucleosides alone is useful in the elucidation of cleavage sites, the determination of the sum of nucleosides + nucleotides leads to a differentiation between the single-hit and the double-hit mechanism. If cleavage occurs along both chains simultaneously, then the analysis of the nucleosides + nucleotides should yield complementarity results, i.e. A = T, and G = C. If cleavage occurs on only one chain, then such complementarity need not be observed. Results with the Cu-DNA reaction show lack of complementarity, thus demonstrating the single-hit mechanism.

C. Book on Inorganic Biochemistry. The complete manuscript is in the hands of the publisher.

Significance to Bio-medical Research and the Program of the Institute. The utilization of the metal inhibited deoxyribonuclease reaction in DNA sequence determination can lead to an understanding of the message in DNA, which contains the genetically determined information for growth, development and aging.

Proposed Course of Project: The success of the reactions of DNAse with Cu-DNA and Hg-DNA, and the importance of obtaining techniques for sequencing DNA, indicate the profitability of extending this technique to the use of other metals. The mechanism of the DNAse reactions modified by metals will be further investigated.

Honors and Awards:
G. Eichhorn was appointed Distinguished Lecturer at the Michigan State University, May, 1972.

G. Eichhorn was invited to present a feature lecture at a symposium on "Inorganic Analogs of Biologically Important Compounds" at the Rocky Mountain Regional meeting of the American Chemical Society, July, 1972.

G. Eichhorn was appointed to the Panel on Nickel, Biological Effects of Atmospheric Pollutants, of the National Research Council.

Publications:
Eichhorn, G. L., "Complexes of Nucleosides and Nucleotides" in: G. L. Eichhorn, (Editor) Inorganic Biochemistry, Elsevier Publishing Co., in press.

Eichhorn, G. L., "Complexes of Polynucleotides and Nucleic Acids", in: G. L. Eichhorn, (Editor) Inorganic Biochemistry, Elsevier Publishing Co., in press.

Serial No. HD-GMA11
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Intermediary Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Control of Metabolism in the Central Nervous System

Principal Investigators: Bertram Sacktor
Meyer S. Hochman
Yoshio Shimada

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.7
Professional:	1.3
Other:	0.4

Project Description:

Objectives: Alterations in the control mechanisms in cerebral metabolism have been initiated in order to understand the deterioration in CNS function with age. Previous studies have shown that hexokinase is a key enzyme controlling the cerebral glycolytic rate. The major portion of the total brain hexokinase, in contrast to that in most other tissues, is localized in mitochondria. Moreover, specific metabolites, at physiological concentrations, can effect the release or rebinding of the enzyme. The physiological significance of this unique localization and the phenomenon of reversible association-disassociation of hexokinase to the mitochondrial membrane are being investigated as a possible critical control of the intermediary metabolism in the central nervous system.

Methods Employed: Current biochemical procedures for brain mitochondrial isolation and measurements of enzyme activities are used, as applicable.

Major Findings: Progress has been made in defining the control mechanisms in cerebral metabolism. Brain mitochondrial hexokinase is reversibly released to the cytosol by ATP and ADP. It has been found that nucleotide debinding is dependent on the ATP:ADP ratio. At a constant total nucleotide concentration, added exogenously, solubilization increased linearly with increasing ratios. At a ratio of 4.8 mM ATP:0.8 mM ADP, the intracellular concentrations in rat brain, 4X as much enzyme was solubilized than at the reciprocal ratio. During oxidative phosphorylation, when ADP is converted to ATP endogenously, the mitochondrial hexokinase becomes solubilized. An

85% increase in the soluble form of the enzyme is found when the physiological concentration of ATP is obtained. This change in compartmentation of hexokinase during coupled respiration, plus the finding that the soluble form of the enzyme is more sensitive to inhibition by glucose-6-P, suggest that the binding and debinding of the enzyme to mitochondria may be a factor in the physiological control of hexokinase activity, and thus the glycolytic rate, in brain.

Significance to Bio-medical Research and the Program of the Institute: The mechanisms of the biochemical control processes in the central nervous system are being established. This information is fundamental to the understanding of the deterioration of CNS function during the aging process.

Proposed Course: Studies are planned to define further the regulatory mechanisms in cerebral metabolism. Possible age-related changes in the control mechanisms will be explored.

Honors and Awards: None

Publications: None

Serial No. HD-AG19(8)
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Intermediary Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Biogenesis and Turnover of Mitochondria and the Mechanism of Action of Endocrines in Regulating Intermediary Metabolism

Previous Serial Number: Same

Principal Investigators: Bertram Sacktor
Bernard Bulos
Walter Greenhouse
Michael Mitchell
Shri Shukla

Other Investigators: Dennis Buetow (Guest Worker)

Cooperating Units: University of Illinois, Department of Biology, Urbana, Illinois

Man Years:

Total:	5.0
Professional:	3.5
Others:	1.5

Project Description:

Objectives: (1) To determine the mechanisms for the biosynthesis, assembly and degradation of mitochondria. (2) To examine the role of hormones and other agents in inducing specific enzymes and in controlling mitochondrial metabolism. (3) To define the mechanisms of the age-related alterations in mitochondrial turnover and in the endocrine regulation of intermediary metabolism.

Methods Employed: Up-to-date biochemical techniques, developed in these laboratories or in the current literature, are used.

Major Findings: The specific activities of the mitochondrial α-glycerol-phosphate dehydrogenase from livers of adult (12-13 months old) and senescent (27-29 months old) hypothyroid (thiouracil fed for 4 weeks) rats have been measured at different intervals following the induction of enzyme synthesis by the administration of a single injection of a physiological amount (20 µg/100 g body wt.) of triiodo-L-thyronine (T_3). There are no significant differences between the two age groups in the rates of induction, the maximal levels of the induced enzyme, or the decreases in activity following the attainment of peak activities. These findings suggest that

the biochemical machinery for protein synthesis in this tissue in aged animals is in no way impaired, at least in this specific model system, and that the livers of senescent rats are fully capable of responding to direct adaptive stress.

Changes in the State 3 (ADP-stimulated) and State 4 (controlled) rates of succinate oxidation in liver mitochondria induced by thyroid hormone have been examined in adult (12-13 months old) and senescent (27-29 months old) rats. Adult animals, made hypothyroid by feeding thiouracil, have significantly decreased State 3 rates of respiration and respiratory control ratios. The State 4 rate of oxidation is not lowered significantly. A single injection of triiodo-L-thyronine (T_3) to these hypothyroid rats results in increases in States 3 and 4 rates of respiration as well as in the respiratory control ratios. The enhancement of the State 3 rate is evident at six hours after hormonal administration, reaching a maximum (2.3-fold the hypothyroid value) in about two days, with this high level being maintained for at least an additional three days. The increase in State 4 rate of succinate oxidation is 1.6-fold; this is attained in one day after injection of T_3 and is maintained for at least an additional four days. The rates of induction by T_3 of the increases in States 3 and 4 rates of succinate oxidation and respiratory control ratio as well as the maximal levels achieved with hormonal administration are the same in adult and senescent rats. These findings suggest that the biochemical machinery necessary for the stimulation in respiratory rates and respiratory control ratio in the livers of aged animals is fully competent in response to thyroid hormone.

Alternative hypotheses on the mechanism of turnover of rat liver mitochondria, e.g., by a dynamic process in which the organelle is assembled from multiple subunits, each turning over at an unique rate, or by a process of replication and degradation as a single unit, are being tested. The different mitochondrial components, such as the outer and inner membranes and the matrix have been isolated and enzymes characteristic of each fraction are being estimated, isolated and purified. Synthesis and degradation (incorporation and loss of radioactive amino acids) of each protein is to be followed. The marker enzymes include monoamine oxidase for the outer membrane, α-glycerolphosphate and succinic dehydrogenase for the inner membrane, and malic dehydrogenase, glutamic dehydrogenase and Δ-aminolevulinic acid synthetase for the matrix. Concomitant measurements of the turnovers of the major phospholipids of the mitochondrial membranes, specifically phosphatidyl choline, phosphatidyl ethanolamine and cardiolipin, are being made. The biosynthetic system in the mitochondria is perturbed by the induction of specific enzymes, α-glycerolphosphate dehydrogenase by thyroid hormone and Δ-aminolevulinic acid synthetase by allylisopropylacetamide.

The role of the autonomous, mitochondrial protein synthesizing system in mitochondrial biogenesis and turnover is being examined. Progress has been made in the development of techniques for the isolation for the first time of polysomes from mammalian mitochondria. A preparation of rat liver mitochondria essentially free of the endoplasmic reticulum has been achieved by a new method involving treatment with digitonin. The RNAs extracted from the pure mitochondrial preparations are being characterized by sucrose density gradients and other techniques.

Significance to Biomedical Research and the Program of the Institute: This study will provide information on the age-related alterations in control systems of intermediary metabolism.

Proposed Course: Research will continue to define the mechanisms of mitochondrial turnover, hormonal induction, and age-dependent changes in regulatory systems.

Honors and Awards:

Bernard Bulos was invited to present a seminar to the Department of Physiological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland

Publications:

Bulos, Bernard, and Sacktor, Bertram: An assay for phospholipase A activity of snake venom with the use of asolectin as substrate. Anal. Biochem. 42: 530-534, 1971.

Bulos, Bernard, Shukla, Shri, and Sacktor, Bertram: The rate of induction of the mitochondrial α-glycerolphosphate dehydrogenase by thyroid hormone in adult and senescent rats. Mech. Ageing Develop., in press.

Bulos, Bernard, Shukla, Shri, and Sacktor, Bertram: Effect of thyroid hormone on respiratory control of liver mitochondria from adult and senescent rats. Arch. Biochem. Biophys., in press.

Serial No. HD-AG20(8)
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Intermediary Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Mechanism of the Decrease in Maximal Work Performance During Senescence

Previous Serial Number: Same

Principal Investigators: Bertram Sacktor
Bernard Bulos
Shri Shukla
Yoshio Shimada

Other Investigators: Stephen O'Brien (Guest Worker)

Cooperating Units: None

Man Years:

Total:	3.0
Professional:	1.5
Others:	1.5

Project Description:

Objectives: Muscular work performance decreases markedly during aging. In a model system, the flight of insects, the duration of flight, and wingbeat number and frequency decline with age. Previous studies have shown that muscular activity is dependent on the control of carbohydrate metabolism. The mechanisms of regulation at different critical control loci are examined in order to determine the basis for the decrement in excitation-contraction-bioenergetic coupling that is found during the aging process.

Methods Employed: Procedures and techniques of modern biochemistry are modified or developed, as applicable.

Major Findings: A correspondence between a decline in the ability of the aged insect to fly and an *in situ* ultrastructural deformation of the flight muscle mitochondria with decreased cytochrome oxidase has been shown previously. The bioenergetic properties of mitochondria isolated from mature and senescent flies have been further characterized. Studies showing significant decreases in the maximally stimulatable respiratory rate and respiratory control in mitochondrial preparations of aged flies have been completed. The resting rate of O_2 uptake and the phosphorylation efficiency are not altered with age. It has been determined that the decrease in

State 3 rates of oxidation of both α-glycerolphosphate and pyruvate+proline is not due to a decrease in the affinity of the system for the substrates, ADP or Pi, as the respective apparent Km values are not altered with age. In addition, it has been found that the apparent Km values for ADP and Pi in the oxidation of pyruvate+proline by flight muscle mitochondria are considerably greater than the values calculated for the oxidation of α-glycerolphosphate. This requirement for high concentrations of ADP and Pi for the maximal rate of oxidation of pyruvate+proline may be explained by the action of these effectors at the dehydrogenase level, rather than at the coupled phosphorylation region.

A study has been initiated on the genetic regulation of bioenergetic pathways during development and senescence in Drosophila. A line of flies has been obtained which has chromosomes deficient at the α-glycerolphosphate dehydrogenase-I locus (II-20.5), the structural gene for the cytosolic NAD-linked α-glycerolphosphate dehydrogenase. The line is maintained as a heterozygote in conjunction with a balanced lethal autosome (SM 1). Individuals homozygous for mutant alleles of α-glycerolphosphate dehydrogenase-1 have negligible levels of the enzyme. Such flies can not fly and have severely reduced competetive viability. When these flies are bred with each other for 10 generations, the progeny regain the ability to fly albeit their α-glycerolphosphate dehydrogenase level remains low. These results suggest a genetic and/or physiological adaptation to permit other enzymes to provide an alternative system to the interrupted α-glycerolphosphate cycle. A comprehensive survey of the enzymes which compensate for the lack of this vital pathway is in progress and the genetic control of such compensation is to be examined.

Significance to Bio-medical Research and the Program of the Institute: The biochemical mechanisms for controlling the metabolism of muscle are being established to provide the fundamental information needed to understand the decrements in the excitation-contraction-bioenergetic processes with age. Also, the programmed developmental sequence of gene action is being studied to provide a prototype for the deterioration of gene expression with aging.

Proposed Course: Studies are planned to define further the mechanisms of regulation in this model system and to examine alterations in metabolism during the aging process.

Honors and Awards:

Dr. Sacktor was invited to present a lecture on The Control of Enzyme Activity at a Symposium on Energy Transformation in Cells, 9th International Congress of Gerontology, Kiev, USSR.

Publications:

Sacktor, Bertram, and Shimada, Yoshio: Degenerative changes in mitochondria of flight muscle from aging blowflies. J. Cell Biol. 52: 465-477, 1972.
Bulos, B., Shukla, S. and Sacktor, B.: Bioenergetic properties of mitochondria from flight muscle of aging blowflies. Arch Biochem Biophys (in press).

Serial No. HD-AG23(8)
1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Intermediary Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mechanisms of Cellular Transport

Previous Serial Number: Same

Principal Investigators: Bertram Sacktor
Russell Chesney
Indira Joshi

Other Investigators: Arnost Kleinzeller

Cooperating Units: Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

Man Years

Total:	4.4
Professional:	2.2
Others:	2.2

Project Description:

Objectives: Physiological systems, such as renal function, which are intimately dependent on transport of ions and metabolites across cellular membranes, show marked functional changes with age. The paucity of information on the enzymatic mechanisms for the transport of these vital cell constituents frequently precludes a full explanation of observed alterations. Investigations on the enzymatic basis for the active transport of carbohydrates and ions are designed to provide this needed information.

Methods Employed: Modern biochemical procedures including enzyme kinetics and purification, column and paper chromatography, radioisotopes, subcellular fractionation, histochemistry and electron microscopy are used.

Major Findings: Studies on the mechanism of active transport in renal tubules have advanced significantly and several basic principles have been established. The rate of binding of D-glucose to the isolated luminal (brush border) membrane, the first step in the transmembranal transport, is dependent on the sugar concentration. Two binding sites have been identified. One, a high affinity site, is saturable at 1-2 μM, with a Km of 0.6 μM and a Vmax of about 1.5 pmoles/min/mg brush border protein. At this site approximately 7×10^{11} molecules of glucose are bound/min/mg protein. The other, a low affinity site, has a Km some 3×10^{4} higher, 20 mM, and a Vmax of 20 nmoles/min/mg protein.

Binding of D-glucose, in contrast to the complete transport system, does not require Na^+. Choline chloride or lithium chloride can substitute for sodium chloride, although the Tris salt is somewhat inhibitory. Ouabain, a known inhibitor of Na^+K^+ATPase and glucose transport in renal slices, does not influence glucose binding.

The importance of sulfhydryl groups for transport is indicated by the inhibition of binding by the diuretic, Mersalyl, and N-ethyl maleimide.

Phlorizin inhibits competitively at Site I (Ki = 0.02 mM), but non-competitively at Site II.

The bindings of sugars exhibit stereospecificity. L-glucose is bound at a rate 20% that of D-glucose, but by a different mechanism. Bindings of the two isomers are mutually independent; additive; L-glucose does not reverse D-glucose binding; and phlorizin does not inhibit L-glucose binding.

D-galactose binding is biphasic, indicating high and low affinity sites and having, respectively, 50 and 25% the rates of D-glucose. Common binding sites for D-glucose and D-galactose are indicated by mutual competitive inhibitions.

Two binding sites for phlorizin have been found, with Km values of 8 μM and 2.5 mM. Only binding at the high affinity site is Na^+ dependent. Phlorizin binding is inhibited in a competitive fashion by D-glucose. When glucose binding takes place in a Na^+-free system there is no inhibition by phlorizin in the high affinity range, although glucose binding is inhibited by the same concentration of phlorizin in the presence of Na^+. These results are consistent with the concept that phlorizin and glucose are bound at sites near to one another, to account for the mutual competitive types of inhibition, but are bound to separate sites, one Na^+ dependent, and the other Na^+ independent.

Various procedures to solubilize the membranous sugar binding factor, including treatment of the isolated brush borders with ionic and non-ionic detergents, lipophylic solvents, proteolytic enzymes, and other chaotropic reagents, have been tried and evaluated. Several proteinaceous and glycolipid fractions, retaining bound glucose, have been separated. Characterization of these fractions is in progress.

Significance to Biomedical Research and the Program of the Institute: This fundamental information is required to define the biochemical mechanisms of transport, which will then be used in studies of the age-dependent changes in transport systems.

Proposed Course: Studies will continue to define the mechanism of active transport.

Honors and Awards:

Dr. Sacktor was invited to present a lecture on sugar transport to a Symposium on "The Biochemistry of the Glycosidic Linkage," San Carlos de Bariloche, Argentina.

Dr. Sacktor was invited to present a seminar on the mechanism of renal sugar transport at National Institutes of Health, Bethesda, Maryland

Dr. Sacktor was invited to present a seminar on the mechanism of renal sugar transport at the Department of Physiological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland

Dr. Chesney was invited to present a seminar at the National Institutes of Health, Bethesda, Maryland.

Dr. Chesney was invited to present a seminar at the Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.

Publications:

Sacktor, Bertram, and Wu, Niou-Ching: Studies on the spatial relationship between intestinal disaccharidases and the phlorizin-sensitive transport of glucose. Arch. Biochem. Biophys. 144: 423-427, 1971.

Sacktor, Bertram: The function of trehalase in the active transport of glucose in the mammalian kidney. In Proceedings of the 2nd PAABS Symposium, Sociedad Argentina de Investigacion Bioquimica, San Carlos de Bariloche, Argentina, in press.

Serial No. HD-GMA4(2)

1. Gerontology Research Center
2. Laboratory of Molecular Aging
3. Intermediary Metabolism Section
4. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Control of RNA Synthesis in Senescent, Developing, and Neoplastic Systems

Previous Serial No.: None

Principal Investigators: David Gillespie (EOD 8/29/71)
Sergio Marshall (Guest Worker)
Bertram Sacktor

Other Investigators: Robert Gallo
Sol Spiegelman
Joseph Gall
W. Carl Saxinger

Cooperating Units: National Cancer Institute, Laboratory of Tumor Cell Biology, Bethesda, Maryland
Columbia University, Department Medical Genetics, New York, New York
Yale University, Department of Biology, New Haven, Connecticut
University of Maryland, Department of Chemistry, Baltimore, Maryland

Man Years:

Total:	0.8
Professional:	0.8
Other:	0.0

Project Description:

Objectives: (1) To test whether senescence correlates with and/or is caused by aberrant steps in information transfer. (2) To examine different systems for the molecular structure of RNAs produced and the relative quantities of the different species. (3) To understand how the phenotypic effects result from changes in genetic control systems.

Methods Employed: The standard techniques of nucleic acid chemistry, biochemistry and biology are used. A primary role is played by techniques of nucleic acid hybridization. Especially important, techniques are being developed to analyze nonradioactive RNA from primary cells.

Major Findings: Message RNA can be identified by the hybridization of its poly A sequences to radioactive poly U. Message RNA levels can be quantitated by measuring the amount of poly U converted to a hybrid structure by a known amount of (bulk) RNA under appropriate conditions. Important results so far obtained are:

(1) Message RNA levels in blowflies drop by a factor of 10 at the midpoint of the adult flies' lifespan. Work is in progress to determine accurately the kinetics of disappearance of mRNA during senescence, changes in the nature of poly A regions, and specific losses of certain message RNA molecules.

(2) RNA-containing viruses which cause cancer can be distinguished from RNA-containing viruses which do not cause cancer, by the amount and length of poly A sequences in the RNA. Of more than 20 viruses studied, no exceptions have been found.

(3) There are sequences in DNA which are complementary to (and code for?) the poly A in RNA. These sequences appear to exist in DNA of double stranded form in chromosomes, a finding which is inconsistent with Crick's recent model for chromosome structure.

(4) Some RNA molecules, apparently ribosomal RNA molecules, contain guanine-rich regions. The amount of these regions is not a constant number and varies, at least, from tissue to tissue in the mouse and from organism to organism. Work is in progress to determine whether the result indicates the existence of multiple species of ribosomal RNA and, if so, whether the sequences are important in genetic control.

(5) RNA-DNA hybridization conditions can be obtained wherein virtually all of a 28S ribosomal RNA input can be converted to a hybrid structure and where over 80% of this structure is resistant to ribonuclease. This result allows us to determine the mistake level in RNA synthesis as previously planned.

(6) A method for carrying out RNA-DNA hybridizations with RNA covalently attached to cellulose or phosphocellulose filter paper has been successfully worked out. The system is now being used for hybridizations using reverse transcriptase DNA products to immobilized RNAs of interest and to isolate human genes in a pure form. The latter objective, while generally useful, will be used by us to isolate appropriate genes to be used as reagents in determining mistake levels during senescence.

Significance to Bio-medical Research and the Program of the Institute: Measurements of amounts and kinds of message RNA from primary cells can shed light on the mechanisms of (genetic) control underlying such phenomena as differentiation, oncogenesis, and senescence.

Proposed Course: To test whether there are any changes in message RNA during senescence.

Honors and Awards:

Dr. Gillespie was invited to present a seminar at the Columbia University, College of Physicians and Surgeons, New York, New York.

Dr. Gillespie was invited to present a lecture at the 25th Annual Symposium on Fundamental Cancer Research (Molecular Studies in Viral Neoplasia), University of Texas, Houston M.D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston, Texas.

Publications:

Gillespie, D., Marshall, S., and Gallo, R.: Tumor virus RNA contains poly A. Nature, in press.

Marshall, S., and Gillespie, D.: A new rifampicin-resistant mutant of E. coli. J. Bacteriology, in press.

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